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PLANT PHYSIOLOGY

OCTOBER, 1945

RESPIRATORY ACTIVITY AND DURATION OF LIFE OF APPLES GATHERED AT DIFFERENT STAGES OF DEVELOPMENT AND SUBSEQUENTLY MAINTAINED AT A CONSTANT TEMPERATURE[1]

FRANKLIN KIDD AND CYRIL WEST

(WITH TWENTY-ONE FIGURES)

In Part I of this series (9) it was shown that the respiratory activity of an apple gathered at "maturity" in the autumn and subsequently kept at a constant temperature was characterized by a rise followed by a fall. This rise in respiratory activity was attributed to a change of state in the protoplasm. It was considered to mark the onset of senescence and was termed the "climacteric." It is interesting to inquire whether the senescent rise occurs in fruit picked in an immature condition.

Outline of the main experiment, 1925

In 1925, Bramley's Seedling apples were taken at intervals during their period of growth from selected trees in an orchard near Cambridge. They were immediately brought into the laboratory and placed in glass containers held at a constant temperature of 12° C. and ventilated by a constant stream of CO_2-free air of constant humidity. Under these conditions the rate of CO_2 production was followed for as long a period as the fruit remained sound; *i.e.*, free from the attack of fungi. The details of the various gatherings are given in table I. The temperatures in the orchard are given in table II.

The glass containers used in these experiments were approximately 700 ml. in volume and were originally designed to hold one fully grown apple. Single apples were the experimental units in the case of the later gatherings. In the case of the earlier gatherings the experimental units consisted of more than one apple (table I). They were ventilated at a rate of about 4 liters an hour, the incoming CO_2-free air being passed through soda lime and through a wash bottle containing a 7 per cent. solution of sodium hydroxide. The number of fruits per container was so arranged that the rate of evolu-

[1] This is the second of a series of papers on the general subject of "Physiology of Fruit."

tion of carbon dioxide per container should be as far as possible the same in all experiments, and in any case not more than 1.5 ml. per hour.

The measurement of carbon dioxide was made by the Pettenkofer tube absorption method, using N/10 barium hydroxide as the absorbent. Subsidiary to the main plan of the research, as stated above, a number of additional experiments of an exploratory nature were conducted.

TABLE I

GATHERINGS OF 1925

GATHERING	DATE	HOURS FROM ZERO TIME	LOT. NO. OF SAMPLES	NUMBER OF APPLES IN SAMPLES	AVERAGE WEIGHT OF APPLE	REMARKS
		hr.			*gm.*	
I	May 26	0	1	6	0.76	
			2	9	0.65	"Cleaned"
II	June 4	216	3	10	2.90	
			4	10	3.18	"Cleaned"
			5	10	3.00	"Cleaned"
III	June 18	556	6	5	20.5	
			7	5	21.8	
			8	10	21.2	
IV	June 30	844	9	4	30.4	
			10	4	33.8	
			11	4	33.2	Kept at 22.5° C.
			12	4	32.2	Kept at 22.5° C.
V	Aug. 4	1684	13	2	76.3	
			14	2	70.2	
			15	2	66.5	
			16	1	129.0	
VI	Sept. 2	2380	17	1	80.0	
			18	1	89.1	
			19	1	140.8	
			20	1	124.6	
			21	1	107.8	
			22	1	115.8	
VII	Sept. 17	2740	23	1	110.0	
			24	1	244.7	
			25	1	113.8	
			26	1	118.7	
			27	1	120.3	
			28	1	93.4	
			29	1	55.3	

The measurement of oxygen intake was made by a manometric method. The closed container holding the apple was connected with a similar closed container by a manometer of capillary tubing filled with a solution of eosin in water. The two chambers were similar in size, close together, and shielded from air currents and from radiation. Each container had a tap opening to the air. The bottom of the apple container was covered with a 7 per cent. solution of sodium hydroxide while the sides of both containers were covered with filter paper soaked in this solution. The apple container was also connected by a capillary tube, provided with a stopcock, to a graduated burette filled with oxygen over a 7 per cent. solution of sodium hydroxide.

The whole apparatus was installed in a constant-temperature room at 12° C. In operation the carbon dioxide produced by the apples is absorbed by the sodium hydroxide; the oxygen consumed by the apples causes a fall in pressure. Oxygen is then passed in from the graduated burette until the manometer shows that the pressures in the two chambers are again balanced. In starting an experiment the first reading is taken after putting in the apple and allowing an hour to elapse before closing the taps connecting both the apple chamber and balance chamber to air. The barometric reading is taken at this time and the volume reading on the oxygen burette, with the manometer connecting the balance chamber and the apple chamber at zero. The tap connecting the oxygen burette and apple chamber is then closed. Every ten hours oxygen is run into the apple chamber in order to keep the manometer reading fluctuating about the positive and negative

TABLE II

TEMPERATURES IN THE ORCHARD

HARVESTS AND DATES, 1925	AVERAGE OF MEAN OF DAILY MAXIMUM AND MINIMUM
	°C.
Between gatherings I and II, May 26–June 4	10.9
Between gatherings II and III, June 4–Ju 18	16.9
Between gatherings III and IV, June 18–June 30	13.0
Between gatherings IV and V, June 30–Aug. 4	17.2
Between gatherings V and VI, Aug. 4–Sept. 2	16.1
Between gatherings VI and VII, Sept. 2–Sept. 17	11.4

sides of zero, corresponding to a fluctuation in oxygen concentration in the apple chamber between 18 and 22 per cent. At suitable intervals the manometer is slowly and carefully adjusted to zero and the second, third, etc., readings on the oxygen burette taken. Alternate sets of readings of intake of oxygen and of output of carbon dioxide are obtained with the same material, by transferring it at intervals from the apparatus for the measurement of oxygen intake to that used for the measurement of CO_2 production.

Results of the main experiment, 1925

All the primary data of the determinations of respiratory activity are presented as graphical records. In these records the rates of CO_2 production per unit fresh weight of apple at the time of gathering are plotted against time, every observation appearing as a point. The time scale is given in hours and in some cases also in days from zero time; namely, 9:30 A.M. on May 26, 1925, the hour and day of the first gathering.

FIRST GATHERING

These were "one-gram" apples gathered May 26, 1925. At this stage the fruits have only just begun to swell after the fall of the petals. The stamens

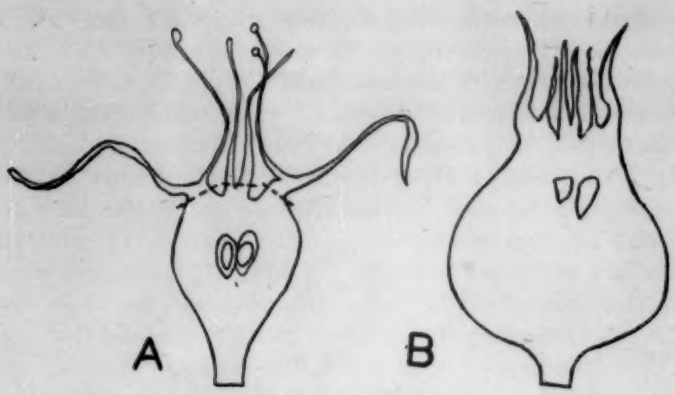

FIG. 1. Diagrammatic median longitudinal section through young apple fruits. Surface hairs not shown. Parts removed from "cleaned" fruits (see text) indicated by dotted lines. A, average weight of fruit 0.7 gm.; B, average weight of fruit 3.0 gm. The same parts were removed in both cases.

persist in a moribund state, dead at their tips, but still alive at their bases. The young fruits are covered with a soft downy coat of fine hairs. Parallel experiments were conducted (a) with fruits intact as gathered (Lot 1); and (b) with fruits from which the downy covering of hairs had been rubbed off and from which the calyx, stamens, and pistil had been removed (Lot 2, "cleaned"). The parts of the fruits removed are indicated in figure 1.

The production of carbon dioxide (fig. 2) was stimulated for a short period by the cutting and cleaning treatment. Subsequent to the first reading, however, the record of the cleaned fruit was 10 to 20 per cent. lower than that of the uncleaned fruit, but similar in form, and continued for a longer time.

The general higher level of the record for the uncleaned fruitlets is most probably due to the fact that in removing the calyx a certain amount of the

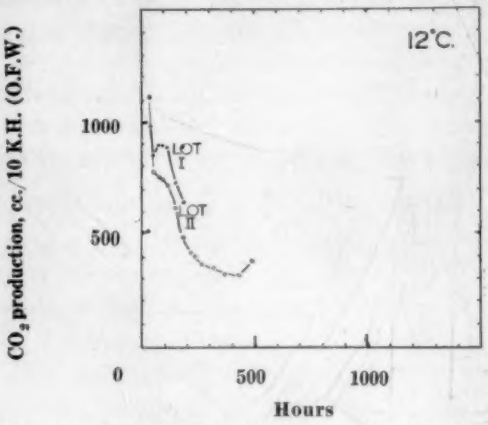

FIG. 2. CO_2 production of Bramley's Seedling apples. First gathering, May 26th. Lot 1, intact fruits; average weight 0.76 gm. Lot 2, "cleaned" fruits; average weight 0.65 gm.

youngest tissue of the growing fruit which is situated at the base of the calyx, was removed. The shorter record obtained in the case of the intact fruit was due to the fact that in their case growth of fungi on the stamen debris became excessive and invasion of the fruit by fungi occurred earlier than in the case of the "cleaned" fruits.

The record for the "cleaned" fruits was continued for 500 hours. The color of the fruits remained green throughout. The final point in the last 100 hours represents a rising trend in respiratory activity which is always associated with invasion by fungi. Several apples of the sample showed mould attack when examined after 500 hours.

This record appears to show two phases. The first phase lasts about 250 hours and is characterized by a rapid fall in respiratory activity broken in its course by a level period or even by a temporary rise. The second phase shows a slow and steady fall in the rate of CO_2 production.

Second gathering

These were "three-gram" apples gathered June 4, 1925. The fruits still possessed a downy covering of hairs and the sepals were inverted and enclosed the dead stamens. Parallel experiments were again conducted (a) with fruits intact as gathered (Lot 3) and (b) with fruits from which the hairs, sepals, and dead stamens had been removed (Lot 4).

A third lot (Lot 5) was used for the measurement of the CO_2/O_2 ratio. The hairs, stamens, and calyx were removed from this lot also.

The "cleaned" fruits (Lot 5) remained sound for about 3,500 hours, and during this period they showed no change of color from green to yellow. The respiratory activity of these "cleaned" fruits was again about 20 per cent. lower than that of the intact fruit (Lot 3). The intact fruit remained sound for only about 1,000 hours.

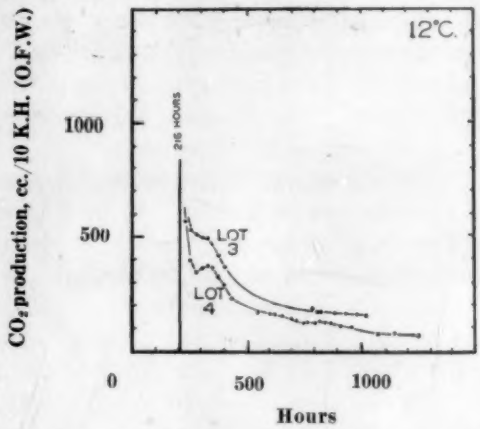

FIG. 3. CO_2 production of Bramley's Seedling apples. Second gathering, June 4th. Lot 3, intact fruits; average weight 2.91 gms. Lot 4, "cleaned" fruits; average weight 3.01 gms.

While the trend of respiratory activity with time is very similar to that shown by the first gathering, the rate throughout is lower at corresponding stages.

The measurements of oxygen uptake show that during the first phase the intake of oxygen closely corresponds to the output of carbon dioxide, the CO_2/O_2 ratio being about 0.9 (fig. 4).

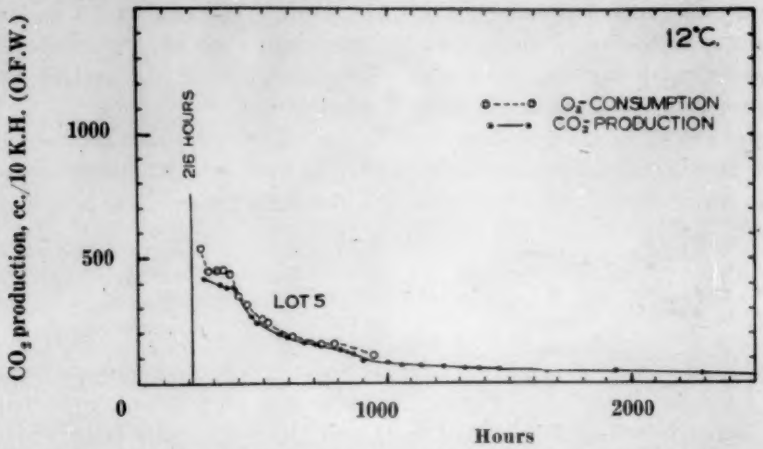

FIG. 4. CO_2 production and oxygen consumption of Bramley's Seedling apples. Second gathering, June 4, 1925; "cleaned" fruits. The measurements of oxygen consumption were discontinued after about 1,000 hours. The rate of CO_2 production showed no measurable change for 1,500 hours beyond the time shown in the figure.

THIRD GATHERING

These were "twenty-gram" apples gathered June 18, 1925. There was now no reason for "cleaning" the apples in any way. Two lots of five fruits (Lots 6, 7) were kept under observation. One of the apples in Lot 6 was attacked by a fungus after about 1,000 hours and was then discarded. All of the apples in the other lot remained sound for about 6,500 hours; *i.e.*, approximately nine months. The last of this group of five apples remained sound for at least 15 months. The apples of this gathering had the longest life of any gathering throughout the experiment.

The trend of respiratory activity at the outset was again very similar to that shown by the previous two gatherings. In the first phase the rise was more pronounced than that occurring in the case of the previous two gatherings. The second phase lasted about 1,200 hours; *i.e.*, until the end of August. A third phase now appeared, lasting 4½ months, during which the steady falling rate was interrupted several times by small rises and falls, each lasting for a short time. At the end of this third phase the fruits were green and sound. The rate then continued to fall steadily (fourth phase) for a further 2,000 hours when a rise in rate of CO_2 production occurred which coincided with the infection by fungi of one of the apples. The loss in fresh weight at this point was 16 per cent. The fruits were still green. The other apples of this lot remained sound for many weeks longer.

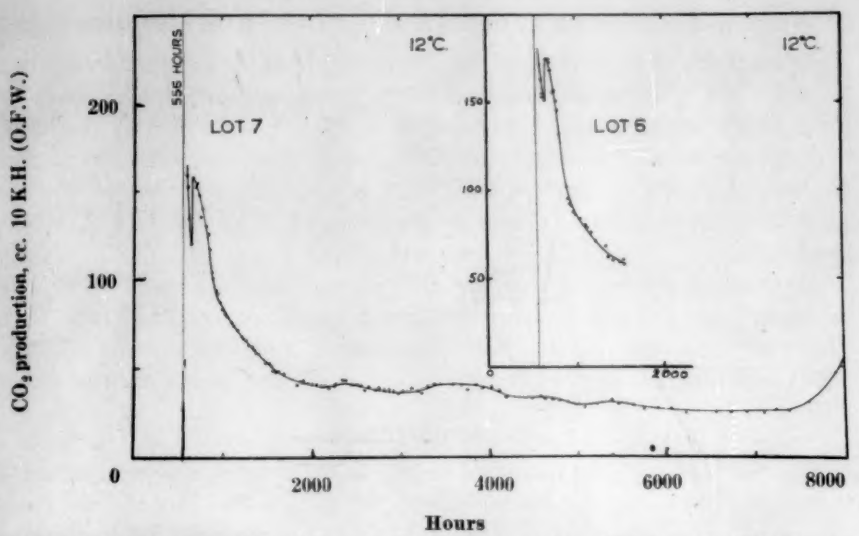

FIG. 5. CO_2 production of Bramley's Seedling apples. Third gathering June 18, 1925; intact fruits.

The series of rises which took place in the third phase are attributed, in the light of the results of later gatherings, to a more or less sudden increase in activity occurring in the case of each of the five individual apples at different times over the period.

As compared with the first and second gatherings the intensity of respiratory activity of the apples of the third gathering was again lower throughout at corresponding stages.

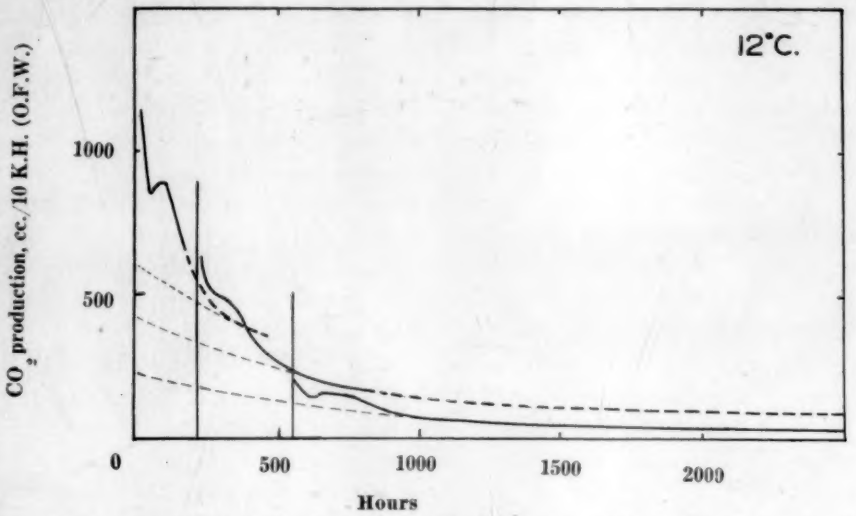

FIG. 6. Respiratory drifts of the first three gatherings (Lots 1, 3, and 7) set out for comparison (diagrammatic).

SUMMARY AND COMPARISON OF GATHERINGS 1 TO 3 OF MAIN EXPERIMENT, 1925

The results of the first three gatherings are brought together in figure 6, in which the plotting of the third gathering is not extended beyond the limits of the second phase. We have, in this figure, suggested by the use of "construction lines" a significant difference in character between the first and second phases in the downward drift of respiratory activity. We consider them to be distinct phases in a process of adjustment to starvation conditions.

A point of interest is that the CO_2 production (expressed as activity per unit fresh weight) of fruit growing normally on the tree, falls off at practically the same rate as that of gathered fruit, the growth of which ceases and which is kept under starvation conditions in the dark at 12° C.

FOURTH GATHERING

The apples have now increased in size to an average weight of about 30 grams; the gathering was made on June 30, 1925. Two lots of four fruits were kept under observation (Lots 9, 10). Lot 9 was used for analysis on August 5, 980 hours after starting the experiment. All apples of Lot 10 remained sound for about 6,500 hours.

A first phase lasting about 350 hours could again be distinguished, but compared with the previous gatherings, there was only a slight indication of a rise in rate during this phase. The second phase of steadily falling respiratory activity lasted about 1,100 hours. This second phase ended only a few days later than in the case of the previous gathering and was succeeded, as before, by a long third phase during which the steady fall in rate was interrupted by a succession of small rises. This third phase was rather shorter than before, lasting 3½ months. A fourth phase of steadily decreasing respiratory activity followed. The first apple of the group to be attacked by molds became diseased about 6,500 hours from gathering. At this time all apples were still green. The loss of fresh weight was about 20 per cent., and the fruits were slightly wilted. Corresponding with the onset of attack by fungi the rate of CO_2 production began to rise.

As regards the intensity of respiratory activity, at the time of picking the fruits of the fourth gathering were less active than those of the third gathering. At the end of the first phase and throughout the second phase, the intensity of respiratory activity of the fourth gathering was practically identical with that of the third gathering at the same time.

Figure 8 shows the results obtained with two lots of fruit (Lots 11, 12) taken after gathering on June 30, 1925 to a temperature of 22.5° C. Lot 12 was first kept under observation. Respiratory activity fell off rapidly for about 140 hours and then increased again even more rapidly. After 350 hours, while the respiratory activity was still increasing, the fruits were discarded, three of the four apples being partly yellow and only one still green. At this time all apples of Lot 11 were still green, and the observation of respiratory activity was continued with these. The curve for the

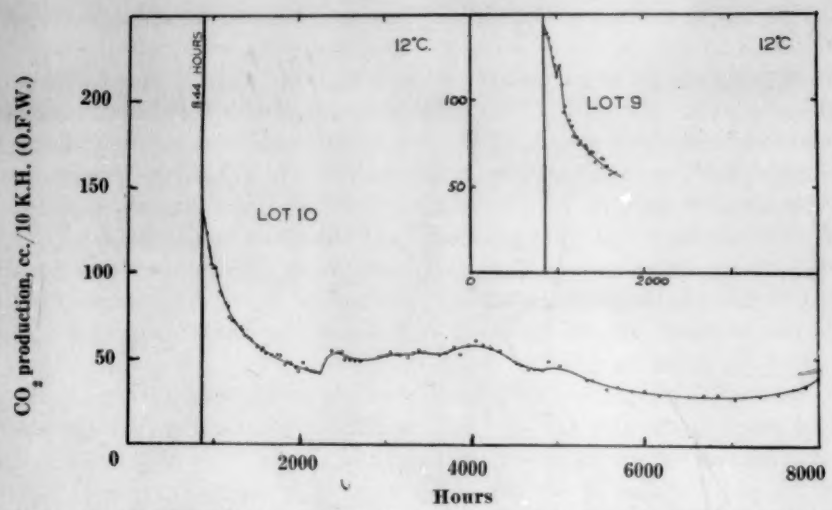

FIG. 7. CO_2 production of Bramley's Seedling apples. Fourth gathering, June 30, 1925; intact fruits.

values obtained fell on the extrapolation of the falling curve of Lot 12. Within a hundred hours or so, however, (550 hours from gathering) the respiratory activity began to rise at first slowly and then very rapidly to the same height as in the case of Lot 12. At the same time three of the apples were yellowing, one being fully yellow and two half yellow at about 1,000 hours from gathering. The remaining green apple was then separated from the others and its respiratory activity followed. Low values were obtained

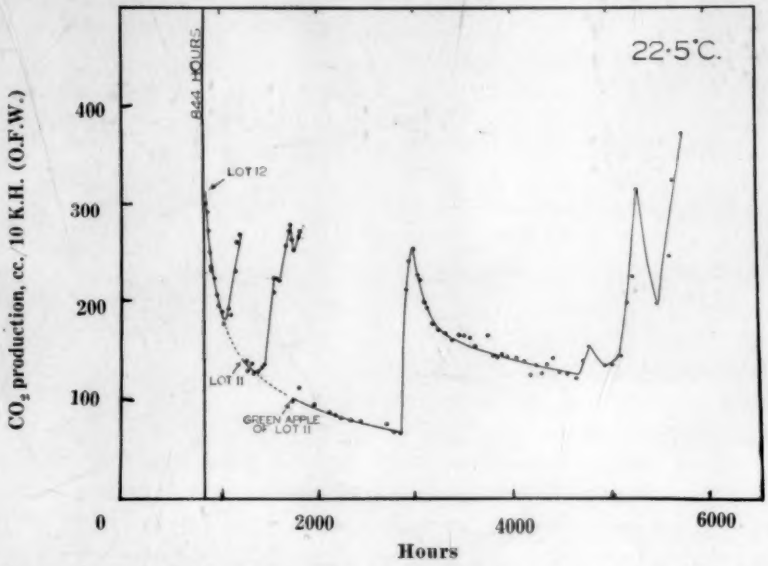

FIG. 8. CO_2 production of Bramley's Seedling apples. Fourth gathering, June 30, 1925; intact fruits (22.5° C.).

and for 1,100 hours a steady fall, appearing as a continuation of the previous falling curves, was registered. Then quite suddenly, within 48 hours, the respiratory activity rose 300 per cent. and continued to rise for another 60 hours to a value nearly four times that recorded before this sudden increase in respiratory activity. During the next 250 hours this outburst of activity partly subsided and the steady falling rate in activity was resumed, but at a higher level, the activity being about three times that which preceded the sudden rise. This fall continued for 1,500 hours before the final rise in CO_2 output, due to invasion by fungi, occurred. It is interesting to note that in this last phase the rate of CO_2 production did not rise steadily but in a series of waves, the maximum of each succeeding wave being higher than that of the previous one.

Of the five phases that have been described in the respiratory history at the lower temperature (12° C.), the first two are not easily distinguishable from one another in this experiment at 22.5° C. A point of interest is that the rate of decrease in activity is identical with that occurring at 12° C. The two curves overlie completely when plotted on the same time scale but with the activity scales in the ratio 2 to 1.

The long third phase in which it was presumed that the respiratory activity of the apples increased individually and more or less suddenly at different times clearly has its counterpart at the higher temperature. Individual variation as regards the length of the period from gathering to this sudden rise was evidently large. The results suggest that in the case of Lot 12, three out of four apples entered this phase simultaneously 250 hours after gathering; that the same happened in the case of Lot 11, but very much later (500 hours after gathering); and finally, they show that the remaining apple of Lot 12 (isolated after 500 hours) did not enter the phase until 2,000 hours from gathering.

The fourth phase is interesting. A single apple only was under observation and showed clearly that the steady fall in respiratory activity of the second phase is continued in the fourth phase, but at a higher level. In the light of more recent work by the authors (**12**) it would appear that in the case of Lot 12 at 22.5° C. the onset of the climacteric in one apple was sufficient to stimulate the onset of the climacteric of others through the ethylene effect. In Lot 11, on the other hand, the first apple to develop its climacteric happened to do so somewhat later than the first apple to do so in Lot 12. Also, in the case of Lot 11, the stimulus failed to affect one of the fruits; this indicates that the concentration of ethylene produced under the conditions of the experiment was very near the threshold value for apples at this stage of development and at this temperature.

Fifth gathering

In this gathering of August 4, 1925, four lots of apples were used. Three of these (Lots 13, 14, 15) consisted of a pair of apples of nearly the same size, each weighing about 70 grams. The fourth (Lot 16) consisted of a

single apple weighing 129 grams. This apple remained sound for 3,700 hours.

The apples of Lot 13 survived 9,500 hours, while those of Lot 14 were sound 7,300 hours after gathering. At this time their loss of fresh weight was 9.2 per cent. and there were signs of slight superficial scalding. Of the two apples of Lot 15, one was attacked by a slowly growing end-rot 5,400 hours after gathering.

The same phases in the drift of change in respiratory activity with time can be distinguished as in the case of the previous gatherings.

In the case of Lot 13 we find an almost continuous fall in the first phase (compare fourth gathering). Lot 16, on the other hand, shows a distinct rise in the middle of this first phase (compare third gathering). The other

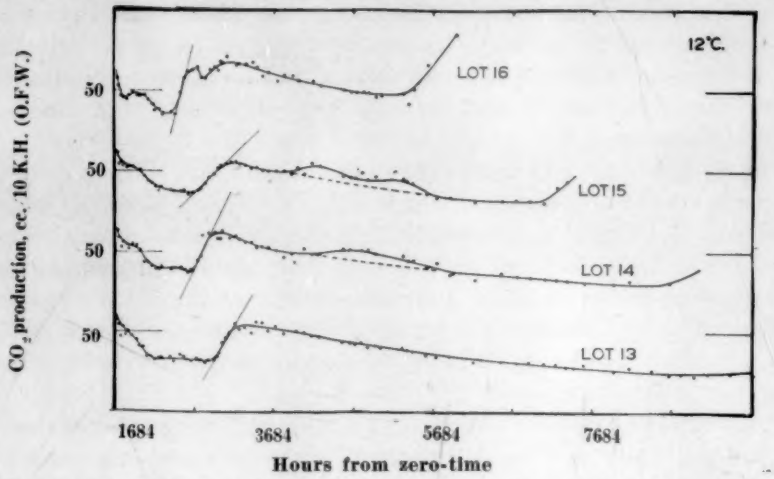

FIG. 9. CO_2 production of Bramley's Seedling apples. Fifth gathering, Aug. 4, 1925; intact fruits.

two lots are intermediate in behavior, showing an approximately level period in the middle of the phase.

The second phase is of much shorter duration than in the case of previous gatherings owing to the fact that the onset of the third phase, marked by a more or less sudden increase in respiratory activity, occurs about the same time as before in spite of the later gathering. Comparing Lots 13, 14, and 15 in which the average weight of the fruits was about 70 grams, there is a difference of about 400 hours between the time of the rise in Lot 13 and the time of the rise in Lot 14; Lot 15 is intermediate. In the case of the larger single apple (Lot 16), the onset of the third phase was considerably earlier (the second phase being almost nonexistent), and the rise in the rate of CO_2 production in the third phase was steeper. The slower rise in the case of Lots 13, 14, and 15 is, we suggest, due to there being two apples which did not synchronize with each other in the time of the occurrence of the rise.

In the third phase there appears to be a definite tendency for irregular

fluctuations in activity to occur. This is seen clearly in the case of Lot 16 (single apple). On general grounds it is not unreasonable to account for these fluctuations by the supposition that the cells of the apple show some variation as regards the time at which the rise in respiratory activity occurs.

When the results of the four lots (seven apples) are averaged and plotted and the resulting curve (fig. 12) compared with that obtained from apples of the third gathering (average of five apples) and the fourth gathering (average of four apples) it is seen that the third phase of respiratory activity, though starting at about the same time, lasts for a much shorter period; *i.e.*, about one month. In other words, there is now less variation among individual apples as regards the time of the onset of the third phase.

The trend of respiratory activity in the fourth phase is the same as in the case of previous gatherings. In the case of Lot 13 the fourth phase lasted over 5,000 hours (7 months). It probably reached its physiological limit, for at the end of this time, although there was no attack by fungi, the rate of respiration ceased to fall and began to rise very slowly with concomitant "scald" development.

In the case of Lots 14 and 15 the steadily falling rate of the fourth phase is interrupted by a temporary, though rather prolonged, period of somewhat higher activity in CO_2 production. This phenomenon is believed to be due to numerous local invasions by fungi the progress of which is checked, so that these invasions result in a surface spotting of the fruit. In the case of Lots 14 and 15 the final rise in rate of CO_2 production is due to the renewed activity of one or more of the checked invasions previously appearing as long-established dry spots.

The initial respiratory activity of the fifth gathering is lower than that of the fourth, but from the end of the first phase onwards the pitch of the respiratory activity of the fifth gathering does not appear to differ from that of the fourth gathering at corresponding points.

Sixth and seventh gatherings

These gatherings were made on Sept. 2 and Sept. 17, 1925, respectively; 2,380 and 2,740 hours from zero time. The sixth and seventh gatherings are considered together (figs. 10, 11). For the sixth gathering there are records for six individual apples (Lots 17–22) and for the seventh gathering records for seven (Lots 23–29). In the case of both gatherings a wide range of size of fruit was taken, but it appears from the records of respiratory activity that size did not affect the drift of respiration, which was determined solely by the age of the fruit.

In the sixth gathering, we again see in its entirety the characteristic first phase lasting 400–500 hours. The second phase has now almost disappeared, the first phase being followed immediately by the more or less sudden rise of the third, or climacteric, phase. In the seventh gathering it seems doubtful whether in any case, except possibly that of Lot 23, the first phase itself is completed before the onset of the third phase. On the average, the third phase begins only 300 hours after gathering.

The sixth and seventh gatherings are the only ones in which there is a full set of records of single apples, so that they are the only ones between which comparisons can be drawn with regard to the steepness and extent of the climacteric rise. There is little difference in the steepness of the rise between these two gatherings and moreover Lot 16 (a single apple) of the fifth gathering also shows the same steep rise. It seems probable, therefore,

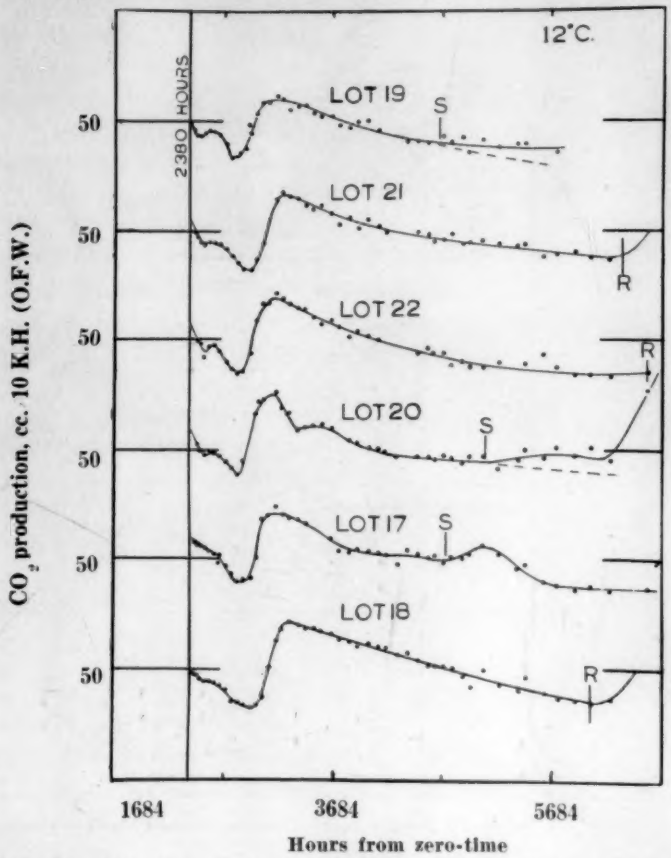

FIG. 10. CO_2 production of Bramley's Seedling apples. Sixth gathering, Sept. 2, 1925. S, skin spotting commenced; R, visible rotting.

that for a single apple the main climacteric rise is always steep. The extent of the rise in activity from the pre-climacteric minimum to the climacteric maximum is nearly 100 per cent. in the case of the sixth gathering as compared with about 70 per cent. for the seventh gathering. The smaller average rise of the seventh gathering may be due to the real extent of the rise not showing itself, because the countervailing causes responsible for the fall in the first phase are still operative at the outset of the third phase in apples of this gathering.

There is no distinct difference between the sixth and seventh gatherings as regards the scatter in time of the onset of the climacteric rise in individual

apples. In both gatherings the scatter is less than that which was shown by the apples of the fifth gathering. For the fifth, sixth and seventh gatherings the onset of the third phase (climacteric rise in respiratory activity) is progressively later in time, but not to an equal extent with the dates of gathering.

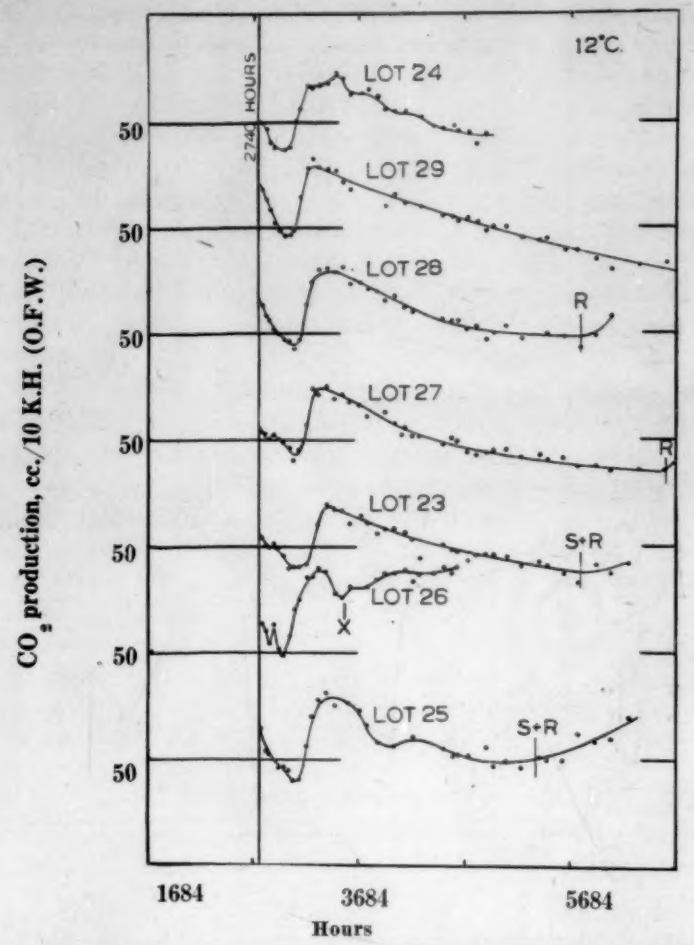

FIG. 11. CO_2 production of Bramley's Seedling apples. Seventh gathering, Sept. 17, 1925. S, skin spotting commenced; R, visible rotting.

Although in these records of single fruits the third phase is considered to consist of one main rise, in some cases there appear to be irregularities in the records both before and after the main rise and, as has been pointed out above, these may be due to variation within the apple as regards the time of the rise for different parts of the tissue. Comparing the one case of a single apple in the fifth gathering, the six cases in the sixth gathering and the seven cases in the seventh gathering, there does not seem to be any marked difference in the duration of the climacteric for a single fruit.

From what has been said above it is clear that the actual duration of this phase for a single cell is a matter of some interest. How far is the slope of the rise a statistical effect? It may well be that for any one cell the change in rate of CO_2 production is extremely rapid.

With regard to the fourth phase, there is no very marked difference between apples of the sixth and seventh gatherings. This phase is shorter in the later gathering. Yellowing of the skin takes place towards the end of this phase and the apples of the later gathering undoubtedly yellowed more quickly than those of the sixth gathering. The downward trend of the fourth phase appears similar to that of the second phase except that it proceeds at a higher general level of activity. The end of the fourth phase is marked by attack by fungi or by functional breakdown (confined to superficial scald in these experiments).

Summary and comparison of gatherings 3 to 7 of main experiment, 1925

The results of gatherings 3 to 7 are brought together in figure 12. In this figure respiratory activity is plotted on a more open scale than in figure 6. The record of the third gathering appears both in figure 6 and in figure 12 so as to illustrate the effect of the change of scale and to coordinate the two pictures. In figure 12, in order to avoid the confusion of overlapping

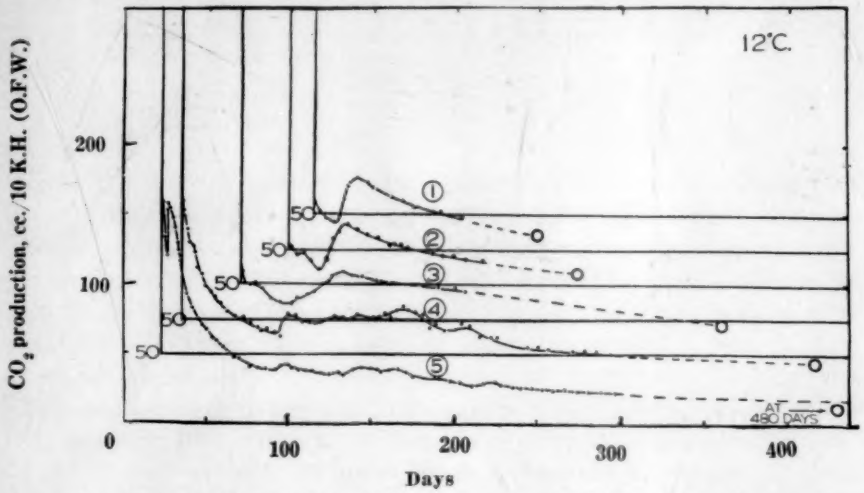

Fig. 12. Respiratory drifts of third to seventh gatherings set out for comparison (diagrammatic). Dotted extrapolations to point in time marked with circle indicate mean life of population. (1) Seventh gathering, average respiratory activity of samples of 6 fruits; (2) Sixth gathering, 6 fruits; (3) Fifth gathering, 7 fruits; (4) Fourth gathering, 4 fruits; (5) Third gathering, 5 fruits.

records, the base-line of zero respiratory activity for each successive gathering has been raised and accordingly horizontal lines corresponding to a respiratory activity of 50 ml. per 10 kilogram hours have been introduced for each gathering to guide the eye. By doing this we tend to obscure the

fact that activity (per unit fresh weight) in fruit growing on the tree, as indicated by the initial determinations of activity immediately after picking, falls in parallel with that of fruit which ceases to grow after picking and is kept under starvation conditions in the dark at 12° C. This fact, however, is brought out in figure 15, in which gatherings 3 to 7 are plotted to a common base-line.

The records are in each case continued to a point in time marked with a circle. This point corresponds approximately to the mean life duration in storage at 12° C. of the population of apples of the gathering in question. For convenience of reference the mean life-duration, the total carbon loss (in terms of hexose) during life after gathering, and the initial respiratory

TABLE III

TOTAL CARBON LOSS (IN TERMS OF HEXOSE) DURING LIFE AFTER GATHERING AND INITIAL RESPIRATORY ACTIVITIES

GATHERING	DATE	INITIAL RESPIRATORY ACTIVITY AT 12° C. CC. PER 10 K.H.	LIFE DURATION AT 12° C.		LOSS OF CARBON (AS GM. HEXOSE/100 GM. FRESH WEIGHT) FROM GATHERING TO DEATH AT 12° C.
			FROM GATHERING	FROM ZERO TIME	
		cc.	*days*	*days*	*gm.*
I	May 26	1400	18	18	3.0
II	June 4	800	146	154	4.2
III	" 18	190	355	377	5.1
IV	" 30	140	333	368	5.0
V	Aug. 4	65	250	321	4.0
VI	Sept. 2	53	167	266	2.5
VII	" 17	62	133	246	2.4

activities of the whole seven gatherings are tabulated in table III and the duration of the different phases as they appear in figures 6 and 12 in table IV.

Progress curves of respiratory activity after gathering reflect an ontogenetic sequence or drift of change. They may be based on a single apple or on groups of apples. We use the term "phase" in the analysis of these progress curves, but it must always be borne in mind that the various characteristics of phases, their duration, are affected by variation in the timing of the sequence; e.g., as between apple and apple, and probably also as between cell and cell. The picture obtained does not therefore represent accurately the ontogenetic sequence of chemical and physical reactions in a single cell. It is the total result of a number of incompletely synchronized, though similar, sequences occurring together.

Figure 12 brings out the fact that in all gatherings, subsequent to the first and second, which did not reach the climacteric phase, the climacteric tended to occur at about the same point in time; and, further, that this point in time approximates to that at which the fruit in the orchard reached maturity. We have seen above that there is individual variation in the time of onset of the climacteric phase and that the younger the fruit when gath-

ered the greater the scatter in time of the onset of the climacteric. The records of figure 12 are records of average activity of groups of individuals, and the effect of the decrease of scatter with age of gathering is seen in the decreasing length of the third phase and the greater and steeper rise in the older fruit. The records of figure 12 show that the length of the period between gathering and the onset of the climacteric phase is for all the gatherings (3 to 7) about equal to the length of the climacteric phase itself; that is, to the extent of the period of individual scatter. Thus individual variation in total pre-climacteric activity as a percentage of the mean may not alter much with gathering age although the absolute scatter in time of the onset of the climacteric does.

The duration of life, whether reckoned from zero time or from time of gathering, markedly decreases from the third gathering (mid-June) to the

TABLE IV

DURATION OF DIFFERENT PHASES

GATHERINGS	TABULATED VALUES OF APPROXIMATE DURATION OF PHASES, FROM FIGURES 6 AND 12			
	1ST	2ND	3RD	4TH
	hrs.	*hrs.*		*days*
I	250	.....	.....	.....
II	300	.....	.....	.....
III	300–400	1200	4½ months	255
IV	350	1000	3½ months	210
V	400–500	200	1 month	220
VI	400–500	.....	15 days	145
VII	300 +	.....	10–12 days	110

seventh gathering (mid-September). This shortening of life duration with extension of the period of growth by cell enlargement seems to be due to a shortening of the fourth or post-climacteric phase. The yellowing of the skin takes place towards the end of this fourth phase and is not associated in time with the third or climacteric phase. Yellowing does not occur for months after the climacteric in the third and fourth gatherings; in the case of the sixth and seventh, it begins to occur much sooner. It thus appears that by remaining on the tree and continuing to grow in size the apple shortens its post-climacteric life. The amount of carbon oxidized from the time of gathering until the end of life also markedly decreases from third to seventh gathering.

CHEMICAL ANALYSES OF FRUITS USED IN THE MAIN EXPERIMENT, 1925

For present purposes, only the results of the determinations made of soluble and insoluble nitrogen need be given (tables V, VI).

The data show clearly that the nitrogen content per unit fresh weight of the apples fell during their growth; that the proportion of the soluble to the insoluble fraction did not vary greatly during growth; and that neither the total nitrogen content nor, in general, the proportion of soluble to insol-

TABLE V

NITROGEN CONTENT PER UNIT FRESH WEIGHT DURING GROWTH ON THE TREE. BRAMLEY'S SEEDLING APPLES, 1925

GATHERING	NO. OF APPLES	AVERAGE FRESH WEIGHT PER APPLE IN SAMPLES ANALYZED	TOTAL NITROGEN PER APPLE	TOTAL NITROGEN CONTENT, PERCENTAGE FRESH WEIGHT	SOLUBLE NITROGEN CONTENT PERCENTAGE FRESH WEIGHT
		gm.	*mg.*	%	%
1	.....	0.70	(3.1)*	(0.45)*	.....
2	12	2.08	5.9	0.284	0.096
3	10	16.60	23.2	0.140	0.042
4	16	31.70	27.3	0.088	0.023
5	12	63.00	25.2	0.040	0.014
6	12	96.00	34.6	0.036	0.012
7	9	99.00	36.6	0.037	0.012

* Assumed on the basis of the results of ARCHBOLD (1), HOWLETT (5), and PFEIFFER (26).

uble nitrogen changed materially during storage at whatever stage the fruit is picked. In the case of the third gathering there was, however, a significant conversion of insoluble to soluble nitrogen during the period immediately after gathering.

The values for respiratory activity per unit nitrogen during growth are given in table VII. For this purpose the values taken for CO_2 production are those for the groups of apples used in each case. There is very little change in activity per unit nitrogen from the third to the seventh gathering. The activity per unit nitrogen of the young fruit, up to three grams weight, is higher.

In figure 13 are plotted the progress curves of CO_2 production per unit nitrogen after gathering of the first, third, fourth, and fifth gatherings, taking the initial activity as unity in each case. These curves are essentially the same in form as those per unit fresh weight since the content of nitrogen

TABLE VI

NITROGEN CONTENT DURING STORAGE

GATHERING	NO. OF APPLES	HOURS FROM ZERO TIME	NITROGEN CONTENT, PERCENTAGE ORIGINAL FRESH WEIGHT		INSOLUBLE NITROGEN, PERCENTAGE OF TOTAL
			INSOLUBLE	TOTAL	
		hrs.	%	%	%
Third	10	556 (gathered)	0.098	0.14	70
	10	1022	0.060	0.13	46
	10	1566 (Lot 5)	0.068	0.15	45
	5	2336	0.077	0.15	51
Fourth	8	844 (gathered)	0.065	0.09	72
	8	1370	0.060	0.10	60
	4	1825 (Lot 9)	0.065	0.12	54
Fifth	12	1684 (gathered)	0.026	0.040	65
	3	2860	0.032	0.044	72
	3	4010	0.031	0.039	79

TABLE VII

RESPIRATORY ACTIVITY (AT 12° C.) PER UNIT NITROGEN CONTENT DURING GROWTH ON THE TREE; GIVEN AS CC. CO_2 PER GRAM NITROGEN PER HOUR

	cc.
Gathering 1	31
" 2	28
" 3	13
" 4	16
" 5	16
" 6	15
" 7	17

does not change during storage. When compared on this basis the results suggest that the fall in activity during the first phase after gathering (for duration of same see table IV) becomes progressively slower and smaller as the age at gathering advances. Just as the duration of the first phase increases, so the time to the end of the inflexion in the first phase likewise increases. In the case of the fifth gathering the climacteric phase occurs immediately after the last phase of adjustment. In the later gatherings which are not shown in figure 13 it supervenes while the first phase is still in progress.

In the earlier gatherings an increase in the sugar content took place during storage which was greater than could be accounted for by the hydrolysis of starch, thus indicating the presence of some other polysaccharide in considerable quantity.

Subsidiary experiments, 1928

Subsidiary sets of experiments were carried out in 1928 and in 1929. The object of those carried out in 1928 was to see whether similar results

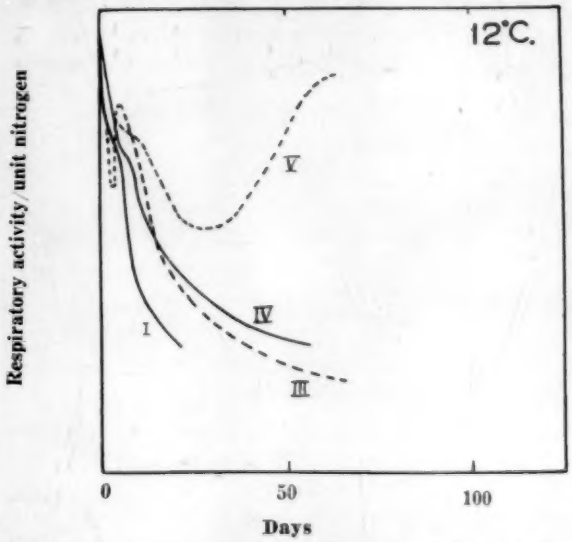

FIG. 13. First, third, fourth, and fifth gatherings compared on the basis of taking activity per unit nitrogen at gathering, as unity.

TABLE VIII

DETAILS OF, AND SAMPLES USED IN, RESPIRATION EXPERIMENT

GATHERING	DATE, 1928	LOT NO.	NO. OF APPLES	AVERAGE WEIGHT	NUMBER OF CONTAINERS	REMARKS
				gm.		
I	May 29	30	5	0.76	One	
II	June 4	31	8	2.2	Two	
III	June 12	32	4	6.4	Two	Air
IV	June 27	33	2	20.0	One	
V	July 11	34	2	29.0	One	
V	" "	35	4	.....	Two	(Nitrogen)
V	" "	36	4	.....	Two	(5% O_2)
V	" "	37	4	.....	Two	(10% O_2)
V	" "	38	4	.....	Two	(50% O_2)
V	" "	39	4	.....	Two	(100% O_2)
VI	July 25	40	4	38.5	Two	
VII	Sept. 3	41a	2	72.5	One	Air
VII	" "	41b	2	72.5	One	
VII	" "	42	2	73.4	One	(5% O_2)
VII	" "	43	2	75.4	One	(5% O_2)
VII	" "	44	4	75.6	Two	(50% O_2)
VII	" "	45	4	74.0	Two	(100% O_2)

would be obtained if the fruit were held, after gathering, at a higher temperature of storage. The temperature employed was 18° C. At the same time experiments were ineluded to study how far the characteristic course of respiratory activity after gathering was dependent upon the concentration of oxygen. The fruit came from the same source as that of the principal experiment.

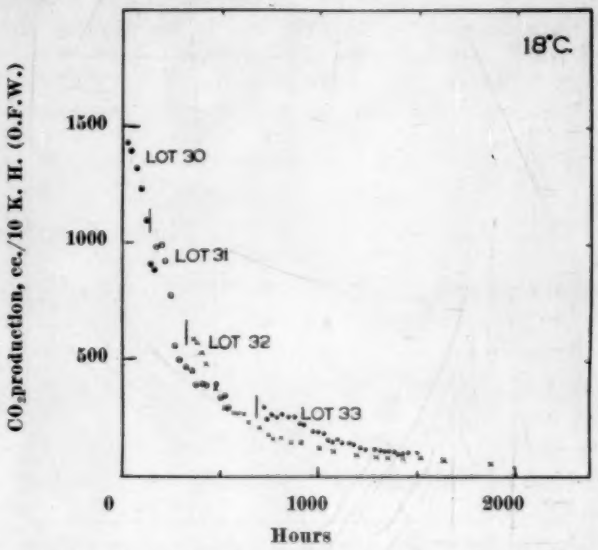

FIG. 14. Records of respiratory activity at 18° C. of fruits of the first, second, third, and fourth gatherings, 1928. The climacteric phase of the fourth gathering, Lot 33, is not included in this figure, but appears in figure 15.

The details of the experiments and of the samples used in the respiration experiments are given in table VIII. The samples of the different gatherings were not kept under observation as long as in the previous experiments and, therefore, the records end arbitrarily while the fruit is still sound. The records of CO_2 production in air for gatherings 1–4, Lots 30–33, are shown in figure 14. Those for gatherings 4, 6, and 7 are shown in figure 15, Lots 33, 40, and 41, and for comparison we have included in this figure a repetition of the 1925 results covering the same period of growth. The record for gathering 5 overlies those of gatherings 4 and 6, and for this reason is not included in this figure. It appears, however, in figure 16.

The identity in behavior as regards respiratory activity per unit fresh weight, of fruit that remains on the tree and increases in size, and of fruit

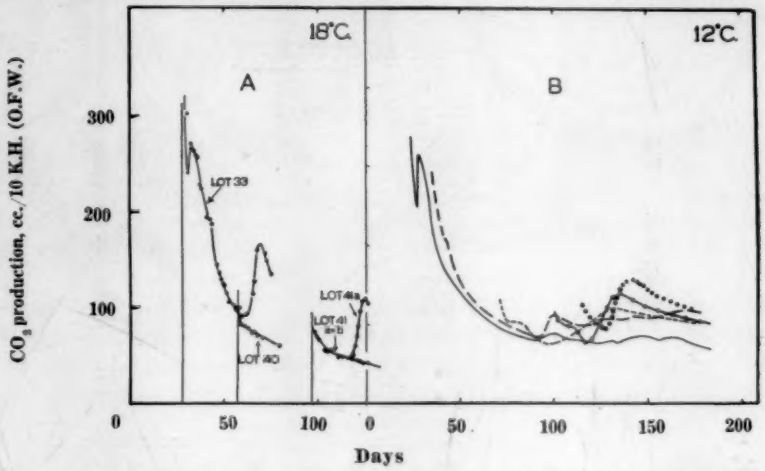

FIG. 15. A. Records of respiratory activity at 18° C. of fourth, sixth, and seventh gatherings, 1928. B. Records of respiratory activity at 12° C. of third, fourth, fifth, sixth, and seventh gatherings, 1925. See figure 12 for ordinate values for CO_2 production.

which is gathered and kept in the laboratory, is again seen. The higher temperature (18° C.) used in these experiments of 1928, as compared with 12° C. used in the previous experiments, does not appear to increase to any marked extent the rate of decrease in activity of gathered fruit. At the higher temperature, however, the downward drift in activity of the second phase appears to proceed towards a lower level.

In most of the gatherings there is an indication of two pre-climacteric phases; the first of these, as in the previous experiments at 12° C., lasting from 10 to 20 days. In the fourth gathering (Lot 33) the first phase appears, as in the previous experiments, to be one of rapidly falling rate interrupted for a time by the operation of some factor tending to cause a rise, before the steady drift of the second phase is reached.

The climacteric phase was not reached before readings were terminated in any of the gatherings except the fourth and the seventh. The time of the onset of the climacteric in the seventh gathering (Lot 41a; gathering date

3/9/28) was almost exactly the same as that of the sixth gathering of the 1925 experiments (gathering date 2/9/25). Lot 41b of the same gathering had not reached the climacteric when observations were discontinued. In the case of the fourth gathering there were only two apples in one container under observation at the time of the climacteric, which occurred about 30 days earlier than in the case of the corresponding gathering in 1925. In these experiments therefore there was no clear indication as to the effect of temperature upon the duration of the pre-climacteric phases.

An effect of one apple upon another in the same container, as regards the onset of the climacteric is again indicated. Clearly the two apples of Lot

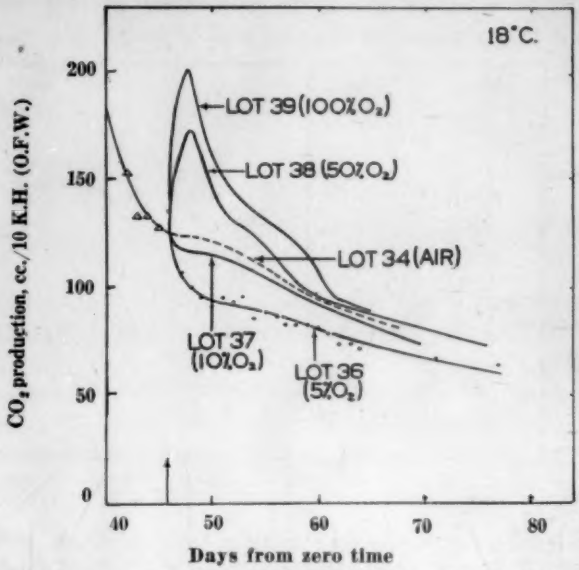

FIG. 16. Fifth gathering, 1928 (18° C.), transferred to atmospheres rich and poor in oxygen, 6 days from gathering. Points before transfer are average of all lots used; points after transfer shown for Lot 36 only.

33 entered the phase almost simultaneously. The same was true of Lot 41a; while Lot 41b, though comparable with Lot 41a in date of gathering and subsequent treatment, still showed no sign of entering the phase 10 days later.

Effect of oxygen concentration

Experiments with the fifth gathering (fig. 16) suggest that oxygen has a more marked effect in the first phase than in the second phase. Supernormal concentrations of oxygen (50 and 100 per cent.) markedly increased activity in the first phase and tended to make this phase contrast more obviously with the subsequent phase of downward drift. Subnormal oxygen concentration (5 per cent.) produced a general lowering of activity and rendered the first phase indistinguishable from the second. When oxygen is reduced to zero concentration the whole character of the phase of adjustment is altered (fig. 17).

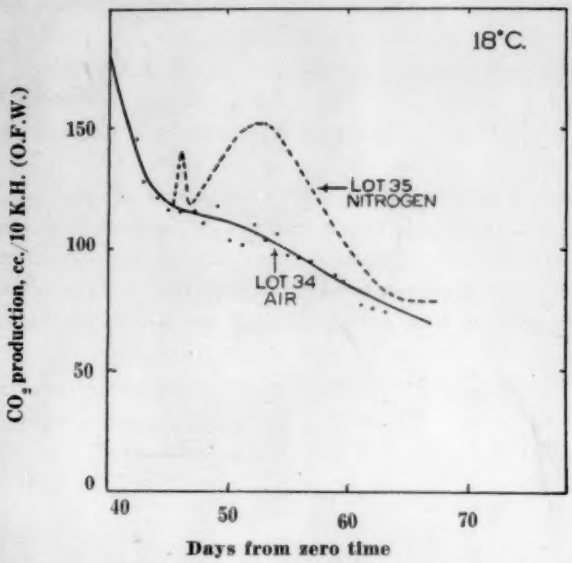

FIG. 17. Fifth gathering, 1928 (18° C.). Lot 35 transferred to atmosphere of nitrogen 6 days from gathering. Lot 34 in air throughout. Points shown for Lot 34 only.

Figure 18 presents the results obtained with the seventh gathering. Transference from air to various oxygen mixtures took place six days from gathering. The concentration of oxygen influenced the time of the onset of the climacteric rise. This began about ten days from gathering in those

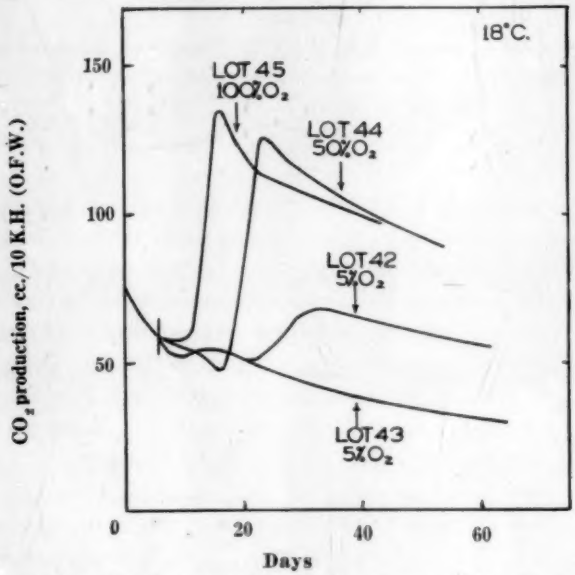

FIG. 18. Seventh gathering, 1928 (18° C.), transferred to atmospheres rich and poor in oxygen, 6 days from gathering.

apples treated with 100 per cent. oxygen; and about 16 days from gathering in those treated with 50 per cent. oxygen. In air, as stated above, one of the lots (41a) began to show a rise in 20 days; the other (41b) had not begun to show a rise by the thirtieth day. In 5 per cent. oxygen one of the lots (42) commenced its rise in 23 days; the other (43) had not shown any rise even after 65 days.

Four apples were treated with an atmosphere of 100 per cent. oxygen, two in each of two respiration containers. The same was the case with those treated with 50 per cent. oxygen. The onset of the climacteric was simultaneous in the two different containers in each case. It would appear, therefore, as if the oxygen was acting directly or indirectly as an over-riding stimulant.

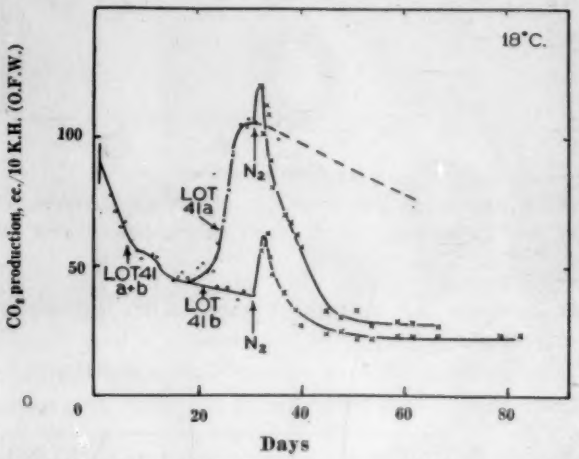

FIG. 19. Seventh gathering, 1928 (18° C.). Transfer from air to nitrogen of Lots 41a and 41b of the seventh gathering on the peak of the climacteric rise, and immediately before the climacteric rise respectively. The interrupted line extrapolating the record of Lot 41a represents the probable drift of activity in air which would have occurred if the transfer to nitrogen had not been made.

There were similarly two lots of two apples kept in air and two lots of two apples treated with 5 per cent. oxygen. Here the results were different. The climacterics were not simultaneous. In both cases it did not occur at all with one pair as long as the experiment lasted. In the case of those treated with 5 per cent. oxygen the form of the curve of the pair showing a climacteric rise suggests that only one apple of the pair entered the phase. Alternatively, if both apples entered the climacteric, it must be concluded that lowering the oxygen concentration to 5 per cent. has a marked effect on the rate of CO_2 production in the post-climacteric phase. That oxygen concentration between 5 and 100 per cent. has relatively little effect on the rate of CO_2 production in the pre-climacteric phase has been noted above in connection with the fifth gathering (fig. 16). This is confirmed by the results with the seventh gathering (fig. 18).

The two lots in air were transferred to nitrogen on the thirtieth day (fig. 19). At this time one was at the peak of its rise; the other, as stated above, had not begun to rise. Immediately on the exclusion of oxygen by the substitution of an atmosphere of nitrogen, CO_2 production in both cases showed a quick rise and thereafter a rather slower fall, this rise and fall being followed by a new steady falling drift. In the case of the pre-climacteric fruit, the new drift in rate of CO_2 production appears practically as a continuation of the drift obtaining before the exclusion of oxygen. In the case of the post-climacteric fruit the fall to the level of the new drift was greater, the level of the new drift in the post-climacteric fruit being in fact almost the same as that of the pre-climacteric fruit.

We have obtained similar results in other experiments (6) which were conducted on Sturmer Pippin apples. In these experiments an additional fact was established; namely, that post-climacteric fruit, on return to air after being kept in nitrogen for three or four weeks, quickly regains its post-climacteric activity.

TABLE IX

GATHERINGS OF 1929

GATHERING	DATE	LOT NO.	NUMBER OF APPLES	AVERAGE WEIGHT
				gm.
I	June 24	46	5	9.5
	" "	47	5	9.5
II	July 9	48	1	21.0
	" "	49	1	23.4
	" "	50	1	21.0

It may be inferred from this evidence that in the absence of oxygen the climacteric does not occur, and further that the exclusion of oxygen after the climacteric has occurred autogenously has the effect of eliminating the influence of the factor responsible for the climacteric rise. Increasing the oxygen concentration above that in air, on the other hand, intensifies the influence of this factor. In the light of more recent knowledge the results might be considered to suggest that the rate of production of ethylene by the apple is a function of oxygen concentration. On the other hand, it is also known that ethylene has no effect upon the rate of CO_2 production of pre-climacteric fruit in an atmosphere of nitrogen from which oxygen is excluded (**12**).

Subsidiary experiments of 1929

The experiments in 1929 were concerned with the question as to how far the striking difference between the third and fourth gatherings in 1925 in the course of respiratory activity in the first phase might be accidental. Fruit was obtained from another source and the details of the two gatherings made are found in table IX. The experiments were carried out at 10° C.

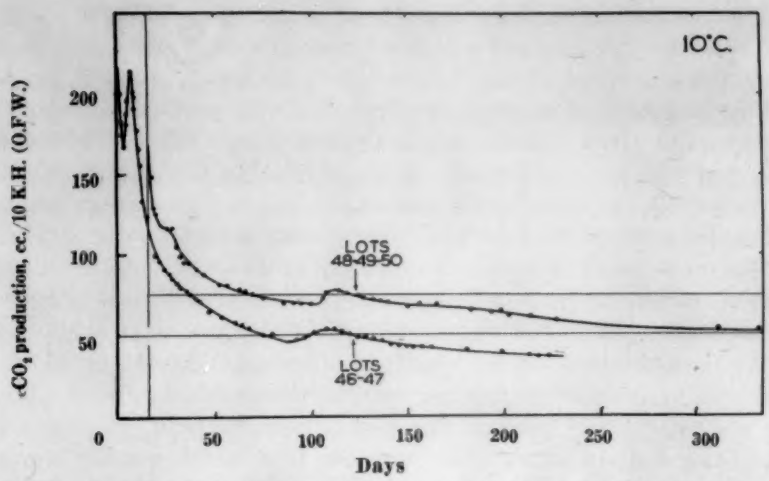

FIG. 20. Records of respiratory activity at 10° C. of two gatherings made on June 29 and July 9, respectively, 1929.

As will be seen from the data which are given in figure 20, close repetition of the results of 1925 was obtained. In the earlier of the two gatherings, the first phase is interrupted by a sharp and distinct increase in activity, while in the case of the later gathering the break in the falling rate of the first phase is marked only by a slight retardation in the fall in activity. It would appear, therefore, that it is only in the particular stage of development which occurs in the middle of June that the course of respiratory activity after gathering shows this marked rise in the first phase. In the 1928 experiments at 18° C. it was the fourth gathering (June 27th) which exhibited this rise.

Data for the change in chemical composition of the fruit after gathering

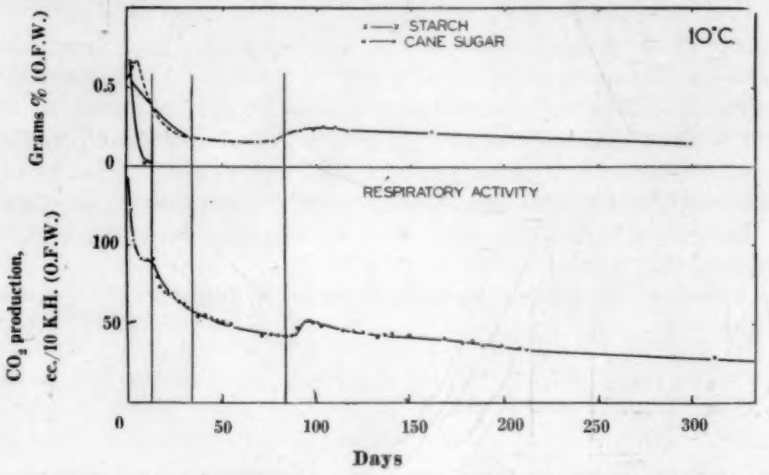

FIG. 21. Starch, cane sugar, and respiratory activity of fruit gathered July 9, 1929. Stored at 10° C.

were obtained for material gathered on July 9th. For the purpose of record, since no other published data for apples at this early stage of development are available, the results are presented in full in table X. For

TABLE X

CHEMICAL ANALYSES OF APPLES DURING STORAGE. BRAMLEY'S SEEDLING APPLES, 1929*

DATE OF ANALYSIS	DAYS IN	RELATIVE FRESH WEIGHT	DRY WEIGHT	TITRATABLE ACID (AS MALIC)	TOTAL SUGAR (AS INVERT) TS	REDUCING SUGAR (AS INVERT) RS	SUCROSE ST—SR	STARCH
STORAGE TEMPERATURE 10° C.								
	days	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*	*gm.*
10. 7.29	0	100.0	.....	1.92	3.89	3.33	0.55	0.60
23. 7.29	13	97.2	11.50	1.96	4.46	4.08	0.38	0.03
12. 8.29	33	94.0	11.11	1.86	4.22	4.05	0.17	0.03
19. 8.29	40	93.5	10.74	1.72	4.20	4.03	0.17	.....
30. 8.29	51	91.5	10.93	1.63	4.08	3.92	0.16	.....
7. 9.29	58	90.3	10.63	1.53	4.02	3.85	0.17	.....
14. 9.29	66	89.6	10.75	1.43	3.93	3.76	0.17	.....
25. 9.29	77	88.1	10.72	1.37	3.97	3.80	0.17	.....
2.10.29	84	87.3	10.35	1.31	3.89	3.69	0.20	.....
8.10.29	90	86.5	10.45	1.32	3.94	3.72	0.22	.....
16.10.29	98	85.6	10.37	1.36	3.75	3.52	0.23	.....
22.10.29	104	84.7	10.02	1.38	3.63	3.39	0.24	.....
29.10.29	111	84.2	9.74	1.09	3.90	3.66	0.24	.....
5.11.29	118	83.7	9.65	1.16	3.64	3.42	0.22	.....
20.12.29	163	78.9	9.37	0.98	3.48	3.27	0.21	.....
6. 5.30	300	58.7	6.83	0.52	2.34	2.20	0.14	.....
STORAGE TEMPERATURE 1° C.								
10. 7.29	0	100.0	.....	1.92	3.89	3.33	0.55	0.60
12. 8.29	33	96.5	.....	1.85	4.44	3.91	0.53	Trace
21. 8.29	42	94.8	.....	1.78	4.38	3.84	0.54	Trace
30. 8.29	51	93.9	.....	1.75	4.39	3.92	0.47	Nil
6. 9.29	58	93.0	.....	1.66	4.08	3.63	0.45	.....
14. 9.29	66	92.0	.....	1.34	4.15	3.75	0.40	.....
25. 9.29	77	91.0	.....	1.47	4.19	3.78	0.41	.....
2.10.29	84	90.1	.....	1.51	4.06	3.68	0.38	.....
8.10.29	90	89.3	.....	1.49	3.88	3.50	0.38	.....
16.10.29	98	88.1	.....	1.45	3.97	3.62	0.35	.....
29.10.29	111	87.4	.....	1.46	4.05	3.70	0.35	.....
26.11.29	139	83.1	.....	1.22	3.67	3.42	0.25	.....
7. 1.30	181	79.0	.....	1.14	3.44	3.22	0.22	.....
1. 4.30	266	72.5	.....	0.83	2.87	2.66	0.21	.....
5. 5.30	314	66.1	.....	0.69	2.69	2.47	0.22	.....

* Number of apples per sample, 50; average weight per apple, 23 gm. Results are means of analyses of duplicate samples expressed as percentage of fresh weight on July 10, 1929. Skin, core, and pips not included in analyses.

present purposes we shall refer only to those relating to starch, cane sugar, and hexoses at 10° C.

These are set out in figure 21 together with the curve for respiratory activity. Starch falls from its initial value of about 0.6 per cent. of the fresh weight to zero in about 12 days; cane sugar falls to a steady value in

about 30 days. This first period of 30 days corresponds approximately in the present case to the duration of the first phase of respiratory activity. The second phase of respiratory activity, the phase of steadily falling rate, lasts about 50 days, and during this period the content of cane sugar decreases only slightly. The climacteric rise which follows is accompanied by a corresponding increase in the content of cane sugar, and the slow fall in respiratory activity in the fourth phase is paralleled by a corresponding slow decrease in content of cane sugar.

In the light of results we have obtained since these experiments herein described were carried out, and which will be reported later, it is certain that during the first few days while starch is rapidly disappearing there was an increase in cane sugar as indicated by the broken line in the figure joining the initial observation to the subsequent observation made on the twelfth day.

The results both as regards content of starch and cane sugar, and as regards respiratory activity, are given in terms of grams per unit original fresh weight.

Discussion

In this discussion references have necessarily been made to observations briefly recorded in the Annual Reports of the Food Investigation Board, London, since the work fully described in the present communication was completed. This paper was written before the outbreak of war. The authors have not been able, until now, to devote the time and attention necessary to prepare it for publication. References to other published work are given up to January, 1940.

The climacteric

In the first paper of this series (**9**) a picture was given of the drift of change in the respiratory activity of Bramley's Seedling apples when gathered at maturity and subsequently stored at three temperatures; namely, 2.5°, 10°, and 22.5° C. This picture has in the present paper been enlarged so as to give a view of the respiratory activity of Bramley's Seedling apples gathered from the tree at various stages of growth and subsequently kept at 10°, 12°, 18°, or 22.5° C. until the onset of functional breakdown or attack by molds.

It is now seen that the climacteric which usually occurs immediately, or almost immediately, after gathering, in fruit gathered in the autumn, can also occur in fruit gathered at any stage during the main period of growth by cell enlargement, which begins about the middle of June when the apple weighs approximately 10 gm. (**29**).

Under the conditions of the present experiments the climacteric showed a tendency to occur about the same time; namely, in the autumn, in the case of all the gatherings which were stored at 10° or 12° C.; i.e., at about the average temperature of the orchard. At higher temperatures (18° or 22.5° C.) the onset of the climacteric usually occurred earlier.

The present writers have shown elsewhere (**11**) that apples which are gathered in the autumn and which have reached the climacteric after gathering, produce a gas which will immediately stimulate the onset of this phase in pre-climacteric fruit, and further that ethylene gas in extremely low concentrations (one part in 1,000) produces an exactly analogous effect. GANE (**2**), in this laboratory, has proved by critical methods of isolation and identification that post-climacteric apples produce small quantities of ethylene.

We have also shown (**13, 20**) that pre-climacteric apples, even when gathered very early during the main stage of growth by cell-division, produce a gas which, if allowed to accumulate, behaves exactly like ethylene. Thus, for example, Bramley's Seedling apples gathered on July 10th at the 30-gm. stage, when kept in a confined atmosphere of air, maintained as regards its composition by chemical absorption of carbon dioxide and by the addition of pure oxygen, immediately show the rise in respiratory activity characteristic of the normal climacteric of mature fruit or of immature or pre-climacteric fruit in a current of air containing traces of ethylene. Similar fruit, ventilated as in the present experiments, did not show the climacteric until the autumn. Measurements of the carbon of volatile carbon compounds evolved by the fruit were made by the authors' soda lime tube and combustion method. The results (**20**) indicated that small but definite amounts of volatile products containing carbon were continuously being produced by the fruit in the pre-climacteric phases and that during the climacteric phase the rate of evolution of such products greatly increased.

More recently various workers (**3, 4, 25**) on apples and pears gathered at about the normal time have carried out measurements of the rate of both CO_2 and ethylene production. Their results show that during the period of the climacteric the rate of ethylene production rises very markedly. Prior to the climacteric the amounts produced are very small.

A tentative conclusion to be drawn from the above facts would appear to be that throughout its growth the apple produces ethylene in very small quantities, but that a minimum or threshold concentration in the tissues is necessary before respiratory activity is affected.

In fruit on the tree or in fruit in storage after gathering, the autogenous climacteric in respiratory activity would then be regarded as ethylene stimulation brought about either by the result of (1) a fall in the threshold value for stimulation, (2) a rise in the rate of production of ethylene, (3) a factor or factors influencing the escape from the fruit of this gas, or (4) a combination of these factors. It is clear, however, that once an autogenous climacteric has occurred, ethylene is produced much more rapidly than before, and for this reason the phenomenon of the normal climacteric appears as an irreversible one.

The results of the present experiments suggest that when small groups of very immature apples were held together under conditions of ventilation with a slow current of air at moderate temperatures (12° C.), an autogenous climacteric occurring in one did not produce sufficient ethylene to stimulate

the others (third, fourth, and fifth gatherings, 1925). At higher temperatures, however [see experiment conducted at 22.5° (1925) and at 18° (1928)] it appeared that the climacteric occurred (with one exception) more or less simultaneously in the case of small groups of immature apples held together in a slow current of air in respiration containers. Hence we may reasonably suppose that at the higher temperatures sufficient ethylene was produced by one apple to stimulate a neighboring apple under the conditions of ventilation employed. We have found, as recorded elsewhere (**12**) with more mature apples of a different variety (Sturmer Pippin at 15° C.), that the climacteric phase appeared earlier, that the rising curve of respiratory activity was steeper, and that the peak reached was higher in the case of a group held together in a single respiration container, than in the case of a corresponding group ventilated at the same rate per apple, each individual of which was isolated in its own small respiration chamber. In the case of the group held together, we appeared to be dealing with conditions under which the ethylene produced by the first apple to reach its climacteric was sufficient to stimulate at once the onset of the climacteric in the other members of the group.

It seems possible to suggest an analogy between the behavior of apples confined in a group and the behavior of cells making up the tissue of a single apple. When one cell undergoes autogenous stimulation and its rate of ethylene production is thereby increased, the concentration of ethylene will correspondingly be raised around it. This in turn may bring about the stimulation of other cells which will then produce more ethylene, until all the tissues of the apple are affected. The observed acceleration of carbon dioxide production, or the duration of the climacteric phase, may, therefore, depend partly on the complex of factors which determine the spread of ethylene stimulation through the tissue; *i.e.*, the degree of ventilation of the internal atmosphere, the variation in the susceptibility of cells to ethylene stimulation, and the extent of increase in rate of ethylene production following stimulation.

If the above picture is a true one the duration of the climacteric phase must be affected by the degree of ventilation of the intercellular spaces of the apple. In the previous paper of this series (**9**) it was shown that the climacteric was very prolonged at low temperatures as compared with high temperatures taking, for example, approximately 190 days at 2.5° C. as compared with 7 days at 22.5° C. The same has since been shown for pears (**16**). At low temperatures the degree of ventilation relative to the rate of production of carbon dioxide and, presumably, of ethylene is greater than at higher temperatures as is shown by the recognized fact that the concentration of carbon dioxide in the internal atmosphere is much lower at low temperatures. The duration of the climacteric at low temperatures may therefore be affected not only by temperature directly, but also by a scatter in time in the stimulation of the individual cells of the apple; and this may be a possible explanation of the high temperature coefficient we have ob-

served for the rate of rise in respiratory activity both in apples and in pears. Our previous view had been that the high temperature coefficient of the climacteric might be accounted for by assuming that some process with a high temperature coefficient, such as protein denaturation, was limiting.

In an attempt to confirm the present suggestion we have shown (**18**) that if single apples (Sturmer Pippins) were taken to a low temperature (1° C.) before the onset of the climacteric phase and were then treated for three days with ethylene (1 in 500 by volume), the climacteric was completed within a much shorter time and the respiratory activity reached a higher maximum value than in similar fruit allowed to develop a normal climacteric at this low temperature.

From what has been said above it follows that the greatest prolongation of pre-climacteric life, other things being equal, might be expected under the conditions most favorable to the escape of ethylene from the fruit. Such conditions would appear to be those of free exposure on the tree. Neither the present experiments nor those recorded in the previous paper of this series include an example of apple left on the tree beyond the time of normal picking maturity, which in the case of the Bramley's Seedling variety grown in England falls definitely in the pre-climacteric phase. It is interesting to inquire, therefore, whether in the case of the Bramley's Seedling variety the climacteric ever occurs on the tree. The impression to be gained from results of experiments covering this point, which have been published in outline elsewhere (**10**), is that the climacteric rise in Bramley's Seedling apples on the tree is a slow, drawn out phenomenon which is accelerated immediately after the fruit is gathered and enclosed in a container for measurement of respiration.

With a late variety (Rome Beauty) (**19**) and with a mid-season variety (Cox's Orange Pippin) we have found that with single fruits, still attached to the tree enclosed in a respiration container through which a current of air was passed, the complete climacteric phenomenon occurred rapidly, just as it did with gathered fruit.

It is interesting to compare the Bramley's Seedling, a late English variety, with early and mid-season English varieties such as the Gladstone and James Grieve. With these varieties results have been obtained (**22**) which show that if the apples are allowed to remain on the tree beyond their normal picking date they reach the post-climacteric stage in respiratory activity. In the case of the McIntosh apple, an American variety, the climacteric occurs just before the normal time of gathering (**27**). The difference in behavior between early and late varieties as regards the occurrence of the climacteric on the tree may possibly be related in part to the larger production of ethylene in the case of the earlier varieties (**24**) which also show greater activity in the production of carbon dioxide (**28**).

As to whether there is any change with age in the threshold value for ethylene stimulation we have at present no direct evidence, and further work dealing with this problem is in progress. There is, however, indirect evi-

dence from the fact that immature pre-climacteric fruit shows recovery from short exposure to ethylene stimulation. After longer exposure there is no recovery (**18**).

The curves of respiratory activity following short period stimulation are extremely interesting. A rapid rise takes place while the stimulus is being applied. As soon as it is removed the curve for respiratory activity falls, but not to the previous level, and finally the autogenous climacteric occurs sooner than it would otherwise do.

It would appear as if different groups of cells had been affected in different degrees; some had been stimulated and had then recovered completely, while others, on the removal of the stimulus, had not recovered. If the stimulus is applied for a sufficiently long time all the cells are affected, and there is no recovery. If these deductions are correct, it follows that there must be a variation in the susceptibility of different cells to ethylene stimulation. Further, the behavior under artificial stimulation in the pre-climacteric phase, contrasted with that shown in the autogenous climacteric, indicates that there must be either a progressive increase with age in the rate of production of ethylene subsequent to stimulation or a fall in the threshold value for stimulation.

One of the most striking results of the present experiments is the fact that there is no marked difference in the time of the onset of the climacteric as between apples gathered when weighing only a few (20 or more) grams and subsequently kept under starvation conditions at moderate temperatures (comparable with those obtaining in the orchard) on the one hand, and on the other corresponding fruit allowed to grow to full size on the tree. It seems obvious to look for some relation between this fact and the equally striking one that there is no marked difference in the rate of decline of respiratory activity per unit fresh weight as between the gathered apples and those remaining on the tree. The two ontogenetic systems appear to be in almost every respect different, physically and chemically. They have, however, two characteristics in common. First, the gross concentration of acid per unit fresh weight declines in both cases to an approximately equal extent, as can be judged by comparing the results of ARCHBOLD (**1**) with those here reported. Secondly, the total production of carbon dioxide per unit fresh weight of tissue is the same in both cases so that one could conceive of a parallel accumulation per unit fresh weight in both cases of some unknown substance produced in the course of respiratory activity. If the climacteric is related either to the fall in acidity or to the accumulation of some unknown substance it would seem most reasonable at present to suppose that these produce their effect by lowering the threshold values of ethylene stimulation. In this connection reference may be made to our experiments (**14**) in which pre-climacteric Sturmer Pippin apples were treated at 20° C. with HCl gas (50 parts per million by volume) and gaseous ammonia (400 to 1,000 parts per million) for two or three days. The ammonia treatment stimulated an immediate climacteric rise; the hydrochloric acid treatment was without effect.

There are other facts with regard to the duration of the pre-climacteric phase in gathered fruit worth noting in this discussion. The first is that with fruit gathered in a very immature condition (**8, 15**); higher temperatures of storage do not always have a clear-cut effect in shortening the pre-climacteric phase. The other is one we have noted both for apples and for pears; namely, that when dealing with commercially gathered pre-climacteric fruit remarkably little difference is observed in the time elapsing before the onset of the climacteric as between fruit kept at low, medium, and high temperatures. The full explanation of these facts is still to be sought, but we feel that there is sufficient evidence to indicate that the explanation may be found in the interplay of the various factors we have indicated as probably operative in determining the onset of the climacteric. As regards the effect of temperature, it may be noted that our results for pears (**21**) have suggested an optimum at 10° C. for the rate of production of ethylene in fruit during the climacteric phase.

Finally, the data on the influence of oxygen and of carbon dioxide upon the climacteric are noteworthy. Investigations here and elsewhere (**7, 12, 23**) reported by us establish that in the absence of oxygen the climacteric does not occur, and suggest the possibility that in the absence of oxygen ethylene is not produced. If the climacteric has occurred, exclusion of oxygen causes a reversion to the pre-climacteric state of activity with the difference, however, that, if oxygen is again given, rise of activity to the post-climacteric level begins at once. This observation could be interpreted on the assumption that, in the absence of oxygen, ethylene continues to be produced by the fruit but cannot exert its stimulatory effect. It has in fact been proved experimentally that the rate of CO_2 production of pre-climacteric apples in an atmosphere of nitrogen is not affected by ethylene. On the other hand, the observation is equally well explained by assuming that in nitrogen the fruits cease to produce ethylene and so revert to a pre-climacteric state. In either case it is implied that the post-climacteric state requires the continuous presence of ethylene for its maintenance.

By raising the oxygen concentration above air values the autogenous climacteric is brought on earlier and the stimulus of one fruit on another is increased. These observations suggest that the rate of ethylene production by the cells of the fruit is a function of the oxygen concentration.

The facts with regard to the effects of carbon dioxide are that moderate concentrations of carbon dioxide (about 10 per cent.) in the atmosphere postpone the onset of the climacteric but do not render the fruit insusceptible to stimulation by ethylene (**12, 17, 18**). In the presence of carbon dioxide the postponed autogenous climacteric is more drawn out and does not show so marked an increase in activity as in the case of a normal climacteric in air, thus indicating a wider scatter in time of the reaction to the stimulus of the different cells of the tissues. These two effects of carbon dioxide suggest that it may act by raising the threshold value of ethylene stimulation.

Carbon dioxide in moderate concentrations in the atmosphere affects the

rate of CO_2 production. In post-climacteric fruit it depresses respiratory activity, but when applied to pre-climacteric fruit during the first phase after gathering, it has the opposite effect, that is, one of increasing the rate of CO_2 production. In the second phase the respiratory activity in the presence of 10 per cent. carbon dioxide is depressed well below the corresponding values in air. Thus in a case of Bramley's Seedling apples gathered on July 10, 1934, with an average weight 30 gm. and placed immediately in an atmosphere containing 10 per cent. of carbon dioxide and 20 per cent. of oxygen at 10° C., the first effect was to increase the rate of CO_2 production above that shown by controls in air. The full depressive effect of carbon dioxide was not shown until 20 days after gathering.

In this experiment the controls in air entered the climacteric phase after about 50 days, while those in the presence of carbon dioxide had not shown any climacteric rise after approximately 120 days. These experiments formed part of an extensive examination of the chemical changes occurring during storage at various temperatures of fruit gathered at a very immature stage of development. The results of this work have not yet been fully published, but it may be put on record here, as it is of interest in relation to what has been said above with regard to acids, that there was no difference in the rate of decrease in the acid content of the tissue as between apples gathered on the above date and stored in air at 10° C., and comparable apples stored in the presence of 10 per cent. carbon dioxide and 20 per cent. oxygen. (See also KIDD and HANES (**7**) who record hydrogen-ion concentrations of sap in this experiment.)

To sum up this discussion of the climacteric phase in the apple, it is suggested that one is dealing with a very subtly balanced phenomenon depending on (1) a slow and possibly steadily increasing rate of production of ethylene during growth, (2) a slow fall with age in the threshold value for stimulation, and (3) a number of variables which affect the rate of escape of ethylene from the fruit. The course of events can be altered by quite small changes in the ventilation of the fruit or by "massing" the fruit. Under extreme conditions cases might be expected where the critical stimulatory effect is never achieved. One such case is recorded elsewhere (**15**), the fruit in question being Comice pears, which not only failed to produce a climacteric but also failed to ripen normally.

CO_2 PRODUCTION, SUBSTRATE, AND ENZYME

In Part I of this series (**9**) the conclusion was reached that the changes in respiratory activity over the climacteric could not be explained in terms of parallel changes in the observed gross concentration of sugars and acids. When viewing the wider picture covering the growth period of the fruit, we must take into consideration changes in amount of cytoplasm present per unit fresh weight as well as changes in the concentrations of substrates. As an approximate measure of cytoplasm the nitrogen fractions of the fruit were estimated. The interesting fact emerged that over the main growth

period of the fruit, that is, the period when growth takes place by cell-enlargement, the respiratory activity per unit nitrogen is a constant. On the other hand, the respiratory activity per unit nitrogen falls sharply immediately the fruit is gathered and separated from its source of supply. On the basis of the classical conceptions of enzyme and substrate it is difficult to bring these two observations into line except by assuming that during growth the concentration of substrate as determined by supply must remain relatively constant. If so, it suggests the operation during growth of an auto-regulatory mechanism. For example, if growth in size of cells and synthesis of protein are functions of respiratory activity, any tendency to increase the concentration of substrate from supply must lead to an increase in R/N and hence to the formation of more protein and larger cells. These results immediately set up counter tendencies towards a lower R/N as the result of consumption of substrate, its dilution by uptake of water, and the increase in nitrogen.

As regards the nature of the substrate, it is sufficient here to state that in gross concentration none of the main components of the fruit, such as glucose, fructose, malic acid, etc., is constant or even approximately constant, during growth (**1**); while, on the other hand, the changes in R/N after gathering do not correspond to the changes in concentration of any of these possible substrates. Between gross concentration of cane sugar in the fruit after gathering, however, and the course of respiratory activity a parallelism exists which breaks down only during the short period of starch hydrolysis when cane sugar is increasing and respiratory activity is decreasing.

Summary

The respiratory activity at normal temperatures of Bramley's Seedling apples gathered at various stages of growth has been examined and recorded.

There is a marked fall in respiratory activity per unit fresh weight during the early stages of the development on the tree—the period of cell division. The fall continues, but much more slowly, during the main growth period—the period of cell enlargement.

The respiratory activity per unit nitrogen, and probably also per unit protein, remains approximately constant during the main period of growth by cell enlargement. During the early stages of growth by cell division the respiratory activity per unit nitrogen is somewhat higher. It is suggested that respiratory activity on the tree is not limited by the effective amount of enzyme present but remains constant as a result of an auto-regulation of substrate concentration, through a linkage between respiratory activity, formation of new cytoplasm, uptake of water, and increasing size of cell.

During both growth periods the respiratory activity, after gathering, per unit fresh weight and also per unit nitrogen, falls.

The drift in respiratory activity per unit fresh weight after gathering shows in general four main phases. These have been discussed in relation to their causation. Particular attention has been devoted to the third or

climacteric phase, and to the effect of temperature, oxygen supply, and ethylene upon its onset.

The climacteric rise in respiratory activity has been shown to occur in fruit on the tree as well as in fruit gathered at any stage of maturity. Evidence is produced and discussed which indicates that the climacteric occurs as a result of the production of ethylene by the fruit and of an auto-stimulation produced by this gas when present in the tissues in amounts above critical threshold values.

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BREAKING THE DORMANCY OF PEACH SEED BY TREATMENT WITH THIOUREA[1]

H. B. Tukey and R. F. Carlson

(WITH FIVE FIGURES)

Introduction

Peach seed (*Prunus persica* Batsch.) requires a period of after-ripening of 10 to 12 weeks at a temperature of 2° to 5° C. before it will germinate (**3**). It has been shown, however, that when peach embryos are excised and placed under conditions favorable for germination, the viable embryos will respond within 7 to 10 days either by germinating or by evidencing some

TABLE I

VARIETIES AND SOURCES OF PEACH SEED USED IN EXPERIMENTS

VARIETY	SOURCE AND SEASON OF PRODUCTION
Ambergem—1	Sparta, Michigan, 1944
Ambergem—2	" " "
Belle	Geneva, New York, "
Champion	" " " "
Early Crawford	" " " "
Elberta—1	" " " "
Elberta—2	Fordsville, Kentucky, "
Halehaven	Geneva, New York, "
Ideal	" " " "
J. H. Hale	" " " "
Lovell— 1	Elverta, California, "
Lovell— 2	Monroe, Michigan, "
Lovell— 3	" " 1943
Lovell— 4	" " 1944
Lovell— 5	Newark, New York, 1943
Lovell— 6	Dansville, New York, "
Lovell— 7	Davis, California, 1944
Lovell— 8	" " "
Lovell— 9	" " "
Lovell—10	" " "
Lovell—11	" " "
Lovell—12	" " "
McAllister	Geneva, New York, "
Muir	" " " "
Naturals—1	Fordsville, Kentucky, "
Naturals—2	" " "
October Elberta	Geneva, New York, "
Polly	" " " "
Salwey	" " " "
Sunbeam	" " " "
"Unknown"—1	McMinnville, Tennessee, 1939
"Unknown"—2	" " 1944
"Unknown"—3	" " "
USSR 106	Geneva, New York, "
USSR 557	" " " "
Ward Late	" " " "

[1] Journal Paper no. 628 of the New York State Agricultural Experiment Station.

degree of separation of the cotyledons. This response has provided a rapid test of germinability of non-after-ripened peach seed (**2, 7, 8**).

The dormancy of some seeds which require after-ripening has been broken by various chemical and environmental treatments. Among these is the breaking of dormancy in lettuce seed by the use of thiourea (**4, 5**). The results reported in this paper deal with the effect of thiourea in breaking the dormancy of peach seed.

Materials and methods

Thirty-four lots of peach pits, including 23 varieties, were used in the tests, as listed in table I. In all, over 10,000 pits were used.

The pits were cracked with a heavy mechanical nut cracker and the seed variously dipped and soaked for different intervals (1, 5, 10, 15, and 30 minutes and 1, 2, 8, 16, 24, 48, and 64 hours) in aqueous solutions of 0.05, 0.1, 0.25, 0.5, 1, 2, 3, 4, and 5 per cent. thiourea. In the seed trade, the seed and the enclosed stony pericarp are called a "seed." In this paper the term "pit" is used to denote the stony pericarp and the enclosed seed, and the term "seed" is used in its botanical sense. Following treatment, the seeds were placed on wet filter paper in Petri dishes and glass-covered, moistened plaster blocks at temperatures favorable to germination (18° to 20° C.).

Results with seed of the Lovell variety

The dormancy of Lovell seed was broken uniformly by treatment with thiourea (**fig. 1**), whereas seed of 22 other varieties either failed to respond

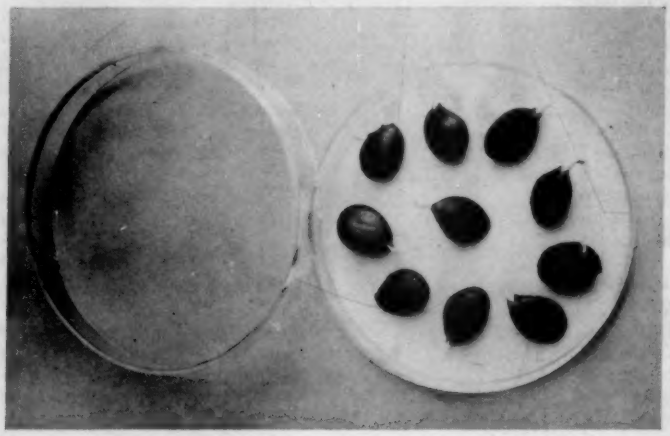

FIG. 1. Germination of non-after-ripened Lovell peach seed following soaking in 0.25 per cent. solution of thiourea for 16 hours.

or responded to a much lesser degree. Within 5 to 7 days following treatment of Lovell seed, the hypocotyl had elongated and had ruptured the integuments, and within 15 to 18 days the epicotyl had elongated and had drawn from the seed coats. The germination and seedling development were

slower than is the case with after-ripened peach seed. The successive steps and the time intervals to reach each stage are shown in figure 2. The seedlings which developed were dwarfish, frequently with anomalous leaves.

The response to different concentrations and methods of treatment are described in detail in the sub-sections which follow.

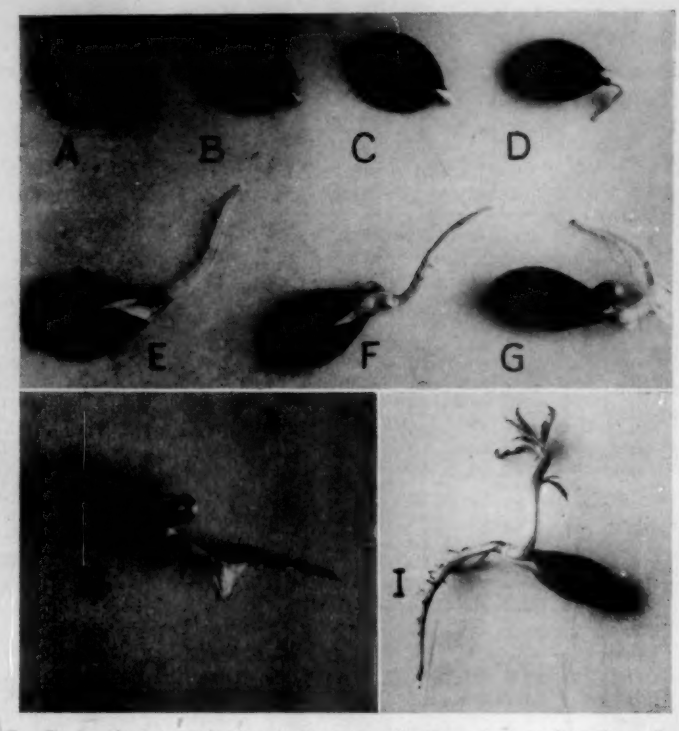

FIG. 2. Successive steps during typical germination of non-after-ripened peach seed, treated with thiourea. Time required to reach successive stages: A, 6 days; B, 7 days; C, 8 days; D, 11 days; E, 12 days; F, 14 days; G, 15 days; H, 16 days; I, 18 days.

EFFECT OF SOAKING LOVELL SEED IN DIFFERENT CONCENTRATIONS OF THIOUREA FOR VARIOUS INTERVALS OF TIME

Lovell seed which was soaked in 0.1, 0.5, 2 and 5 per cent. aqueous solutions of thiourea for 1, 5, 10, 15, 30, 60 and 90 minutes, and for 2, 8, 16, 48 and 64 hours responded most favorably to soaking in 0.25, to 0.5 per cent. for 2 to 16 hours (table II).

At the lower concentrations (0.1 per cent.) a longer period of soaking (48 hours) was required for the same percentage germination as that secured by a shorter period of soaking (2 hours) at higher concentrations (0.5 per cent.). The high concentration (5 per cent.) and long period of soaking (64 hours) was injurious and retarded development of both hypocotyl and epicotyl. The low concentration (0.1 per cent.) caused no injury, even with 48 hours' soaking, whereas 2 and 5 per cent. solutions retarded development following only 2 hours' soaking.

Effect of rinsing Lovell seed after soaking in a solution of thiourea

One lot of Lovell seed which was soaked for 64 hours in 0.5 per cent. thiourea was rinsed in running water for a few minutes before being placed on moist filter paper in Petri dishes for germination. A second lot was not rinsed. The non-rinsed lot was injured by the long soaking, so that only 50 per cent. of the seed germinated, and there was browning of both hypocotyl and epicotyl of those seeds that did germinate. On the other hand, the germination of the rinsed seed was 100 per cent. and no injury was observed.

TABLE II

Germination of non-after-ripened Lovell peach seed after soaking in aqueous solutions of 0.1, 0.5, 2, and 5 per cent. thiourea for various intervals of time

Time	Concentration	Cumulative total per cent. germination					Effect on root	Effect on epicotyl
		Days after treatment						
		6	7	10	12	19		
	%	%	%	%	%	%		
1 min.	0.50	0	10	30	40	40	No injury	No injury
5 "	0.50	10	20	40	60	60	No injury	No injury
10 "	0.50	0	20	40	50	70	No injury	No injury
15 "	0.50	10	20	50	60	70	No injury	No injury
30 "	0.50	10	20	40	60	70	No injury	No injury
60 "	0.50	10	20	40	60	70	No injury	No injury
90 "	0.50	20	30	50	60	80	No injury	No injury
2 hrs.	0.10	0	10	40	40	40	No injury	No injury
2 "	0.50	0	60	80	90	100	No injury	No injury
2 "	2.00	40	50	80	80	80	Retarded	Retarded
2 "	5.00	0	0	60	60	70	Retarded	Retarded
8 "	0.10	20	30	40	50	50	No injury	No injury
8 "	0.50	10	40	50	80	80	Discolored, retarded	Retarded
8 "	2.00	60	60	70	80	80	Discolored, retarded	Retarded
8 "	5.00	0	0	0	30	30	Discolored, retarded	Retarded
16 "	0.25	10	30	50	90	100	No injury	No injury
16 "	0.52	10	30	50	90	100	Discolored, retarded	Retarded
16 "	0.50	10	40	70	80	80	Discolored, retarded	Retarded
16 "	2.00	0	40	80	80	80	Discolored, retarded	Retarded
16 "	5.00	0	0	20	20	20	Discolored, retarded	Retarded
48 "	0.10	0	30	50	70	70	No injury	No injury
48 "	0.50	0	50	60	60	70	Discolored, retarded	Retarded
48 "	2.00	10	10	30	40	40	Discolored, retarded	Retarded
48 "	5.00	0	0	0	0	0	.....	.....
64 "	0.25	0	10	30	40	40	Discolored, retarded	
64 " rinsed	0.25	30	40	60	80	90	No injury	Retarded No injury
64 "	0.50	0	20	30	50	50	Discolored, retarded	Retarded
64 " rinsed	0.50	40	50	100	100	100	No injury	No injury
Control	.....	0	0	0	0	0	.....	.....

Effect of a continuous supply of thiourea during germination of Lovell seed

The favorable results from rinsing Lovell seed in water after soaking for a long period (64 hours) suggested that the response from thiourea might be due to absorption or adherence of thiourea, and that germination might be affected by providing a continuous supply of thiourea during germination. To test this possibility, seeds of the Lovell variety were soaked in water for 16 hours, and then placed in Petri dishes on filter paper which has been moistened with solutions of thiourea of 10 concentrations, ranging from .005 to 5 per cent. A measured amount of material was ad led to each Petri dish; namely, 3 cc. of the desired concentration at the start, and successive amounts of 2 cc., 1 cc., and 1 cc. as needed.

TABLE III

Germination of non-after-ripened Lovell peach seed in a continuous supply of 0.005 to 5 per cent. solution of thiourea

Concentration	Cumulative total percentage germination (days after treatment)											
	6	7	8	9	10	11	12	13	14	15	16	17
%	%	%	%	%	%	%	%	%	%	%	%	%
5.000	0	0	0	0	0	0	0	0	0	0	0	0
4.000	0	0	0	0	0	0	0	0	0	0	0	0
3.000	0	0	0	0	0	0	0	0	0	0	0	0
2.000	10	10	10	10	10	10	10	10	10	10	10	10
1.000	0	0	10	10	10	10	10	10	10	10	20	20
0.500	0	10	20	20	30	40	60	60	60	70	70	70
0.250	10	20	30	40	60	70	90	90	90	100	100	100
0.100	0	10	20	20	20	20	50	50	50	50	80	80
0.050	0	0	10	30	50	50	60	60	70	70	70	70
0.005	0	0	0	0	0	0	10	10	10	10	10	10
Control	0	0	0	0	0	0	0	0	0	0	0	0

The method proved effective, and the results were uniform and consistent. They are given in table III and shown graphically in figure 3. The most favorable response was secured from 0.25 per cent. thiourea. Lower concentrations were less effective, and higher concentrations were injurious. Unless first soaked in water, the seeds germinated slowly and irregularly.

Seed which tested 88 per cent. by the excised-embryo technique, showed 75 per cent. germination without excising when soaked in water for 16 hours and then placed on plaster blocks with a continuous supply of 0.25 per cent. thiourea. Another lot soaked for 16 hours in 0.05 per cent. thiourea and then supplied with a continuous supply of 0.05 per cent. thiourea germinated 75 per cent.

The possibility is suggested of using the thiourea treatment as a rapid approximate-test of germinability of Lovell peach seed without the necessity for excising the embryo. In this connection four lots of Lovell seed were placed on moist blotting paper which had previously been saturated with

0.05 per cent. thiourea and then dried. Germination was less than half that secured from treatments with a continuous supply of 0.25 per cent. solution, yet the possibility remains of adapting thiourea-impregnated blotting paper to this use by using heavier impregnations.

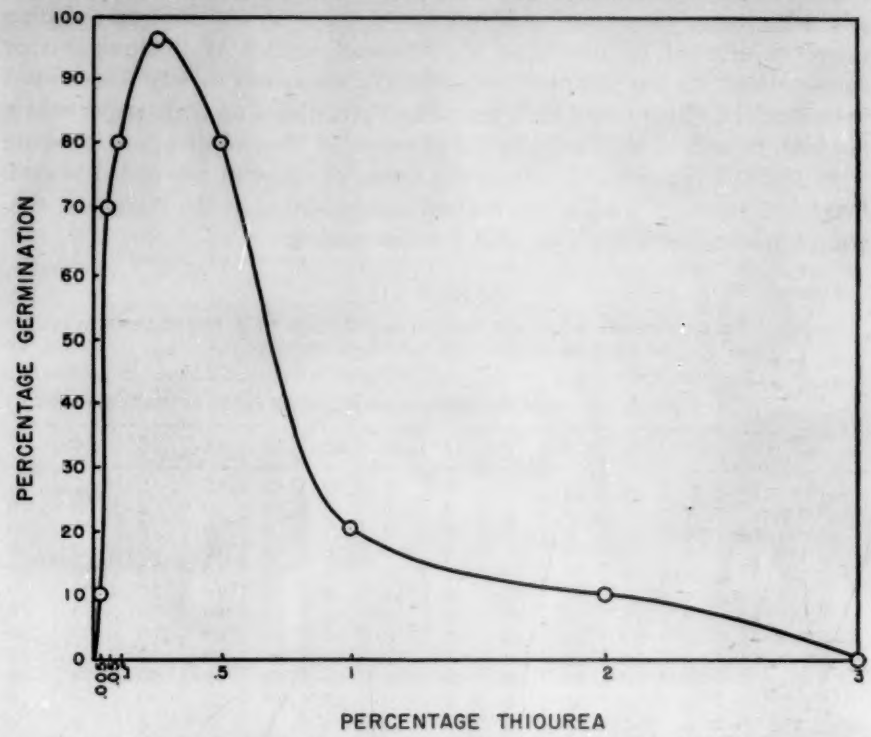

FIG. 3. Effectiveness of different concentrations of thiourea in inducing germination of non-after-ripened Lovell peach seed. All treatments soaked in water for 16 hours and germinated in Petri dishes on filter paper moistened with thiourea solution of the concentrations noted.

EFFECT OF THE STONY PERICARP ON THE GERMINATION OF LOVELL PEACH SEED TREATED WITH THIOUREA

It has been reported in the literature (3) that the presence of the stony pericarp delays or inhibits germination of the seed. Nurserymen commonly crack non-opened peach pits in spring following after-ripening out-of-doors over winter. To determine the effect of the stony pericarp on germination, pits were soaked in 0.25 per cent. solution of thiourea for 16 hours and placed on glass-covered, plaster-block seed germinators which were kept saturated with 0.25 per cent. solution of thiourea. The seed failed to germinate and to crack the pits. Yet when the pits were cracked by hand and the seed placed on plaster blocks saturated with 0.25 per cent. solution of thiourea they germinated. That the thiourea solution reached the seed in uncracked pits was determined by examination. Further, several lots were

de-aerated under partial vacuum so that thiourea solution filled the air space within the stony pericarp, surrounding the seed. Such seed, however, did not germinate unless the stony pericarp was removed.

Appearance of seed coats of after-ripened and non-after-ripened, thiourea-treated Lovell seed

An explanation for the failure of non-after-ripened, thiourea-treated seed to germinate unless removed from the stony pericarp is suggested by a comparison with after-ripened seed. It has been observed that the cotyledons of after-ripened seed increase in size during germination. Not only is the stony pericarp split by the expanding pressure of after-ripened seed but the seed coats also are checkered and ruptured. In contrast, the cotyledons of non-after-ripened, thiourea-treated seed do not enlarge similarly,

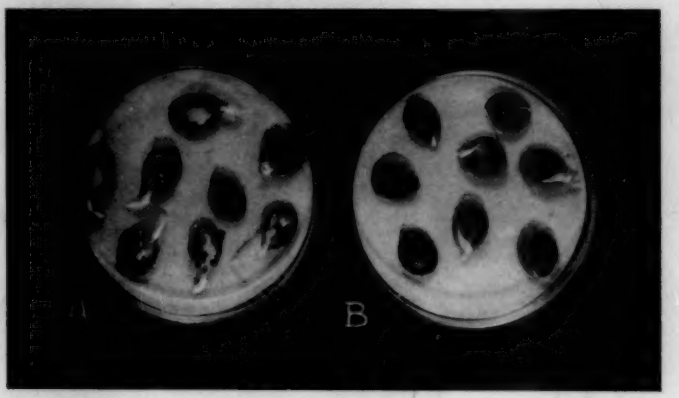

FIG. 4. Appearance of germinating Lovell peach seed: A, after-ripened for 12 weeks at 3° C.; B, non-after-ripened, soaked in 0.25 per cent. thiourea for 16 hours. Note checkering of seed coats of after-ripened seed, A as contrasted with smooth appearance of non-after-ripened, thiourea-treated seed, B.

and the seed coats remain unchanged and unruptured excepting where the hypocotyl breaks through and the epicotyl withdraws from between the cotyledons. Figure 2 shows the germination of Lovell seed in this way. Figure 4 illustrates the checkered appearance of seed coats of germinating Lovell seed after-ripened lots in comparison with the uncheckered appearance of non-after-ripened, thiourea-treated seed.

Characteristics of seedlings from non-after-ripened, thiourea-treated Lovell seed

The seedlings which developed from non-after-ripened, thiourea-treated Lovell seed were dwarfish, with shortened internodes, and anomalous leaf characters (fig. 5). Many hundreds were grown, and all showed this characteristic growth. They were identical in appearance to seedlings grown from excised embryos of non-after-ripened seed (**1, 6, 9**). As with excised embryos, the dwarfish condition was confined to the epicotyledonary axis. New shoots from lateral buds showed "normal" growth characters.

Reduction of fungus infection through treatment with thiourea

In the course of these studies it has been observed that molds and common seed-borne fungi are less prevalent on seed treated with thiourea. The incidence of infection has been reduced greatly by merely dipping seed in solutions of thiourea. Seed has been kept in a germination chamber for 3 months, watered with 0.25 per cent. solution of thiourea without being attacked by molds or fungi.

Fig. 5. Dwarfing peach seedlings with characteristic rosettes and anomalous leaves developing from non-after-ripened, thiourea-treated peach seed.

Effect of thiourea on nine additional lots of Lovell seed

To further establish the specific nature of the Lovell variety in responding to treatment with thiourea, nine additional lots were secured from California, each from a different locality and source. They were received in varying stages of after-ripening, and in different physical conditions. Some had been left out-of-doors, some had been stored indoors, some were cleaned, some were uncleaned. All nine lots germinated without further after-ripening after soaking for 16 hours in a solution of 0.25 per cent. thiourea.

Results with varieties other than Lovell

Seed of 22 other varieties of peaches were treated with thiourea in the concentrations used for seed of the Lovell variety, and for similar varying intervals of time. The comparative tests were conducted in every month

from mid-summer to spring, and were repeated many times. None of the 22 varieties behaved as did the Lovell variety. Whereas Lovell seed from all the different sources germinated readily and similarly after treatment with thiourea, six lots from other varieties failed to germinate at all, and ten others showed only 10 per cent. germination. The highest germination other than with the Lovell variety was one lot of "Naturals" from the Appalachian Mountains which germinated 60 per cent. The results are shown in table IV.

TABLE IV

PERCENTAGE GERMINATION OF VIABLE SEEDS OF SEVERAL VARIETIES OF PEACHES FOLLOWING TREATMENT WITH 0.5 PER CENT. THIOUREA FOR 16 HOURS. GERMINATION BY EXCISED-EMBRYO METHOD GIVEN FOR COMPARISON OF VIABILITY

VARIETY	VIABILITY AS SHOWN BY EXCISED-EMBRYO METHOD	GERMINATION FOLLOWING THIOUREA TREATMENT
SAMPLES OF THE LOVELL VARIETY		
	%	%
Lovell— 1	100	90
Lovell— 2	100	100
Lovell— 4	45	50
Lovell— 5	100	80
Lovell— 9	100	100
Lovell— 8	100	80
Lovell—10	80	70
SAMPLES OF OTHER THAN LOVELL VARIETY		
Ambergem—1	100	20
Ambergem—2	100	10
Belle	100	0
Champion	100	0
Early Crawford	100	30
Elberta	100	10
Halehaven	100	10
Ideal	100	10
J. H. Hale	100	10
McAllister	100	10
Muir	100	10
Naturals—1	100	60
Naturals—2	80	40
October Elberta	100	0
Polly	100	10
Salwey	100	40
Sunbeam	100	0
"Unknown"—1	100	0
"Unknown"—2	100	0
"Unknown"—3	100	10
USSR 106	100	10
USSR 557	100	30
Ward Late	100	0

Discussion

Every effort was made to secure samples of Lovell seed produced in different seasons, grown in different localities, and handled in different ways. The Lovell variety is grown almost exclusively in California and,

regardless of their immediate source, most if not all of the samples of Lovell used in these tests probably originated in that state. The Muir variety is also grown almost exclusively in California, but the seeds of Muir used in these tests were from fruit grown at Geneva, N. Y. Accordingly, it is not thoroughly established that the differences which are reported here for the Lovell variety are peculiar to that variety and to that variety alone. It may be that climate or other factors commonly employed in the production of the Lovell seed by the nursery trade are the controlling factors rather than anything inherent in the variety itself.

Nevertheless, the specific nature of the responses of the different varieties used in these tests suggests the importance of a critical comparison of seed which germinate following treatment with thiourea and seed which do not. The procedure suggests an additional approach to the study of dormancy and after-ripening.

In the complete after-ripening of peach seed, physiological changes occur which result not only in the breaking of dormancy but also in the development of typically vigorous peach seedlings. In another paper the authors have shown that incomplete after-ripening may result in the breaking of dormancy, but that the seedlings which develop may be dwarfed and have anomalous leaves (**9**). The breaking of dormancy of Lovell seed by treatment with thiourea is similar to the breaking of dormancy by incomplete after-ripening. Although the seed may germinate, the seedlings are typically dwarfish, with anomalous leaves.

The data show that the after-ripening processes of peach seed are more complex than the simple removal of an inactivator or an inhibitor from the seed coats. The failure of cotyledons of non-after-ripened seed to swell and to rupture the seed coats and the stony pericarp are evidence in this direction.

Summary

1. Thirty-four lots of peach seed, including 23 varieties, were treated with various concentrations of thiourea in aqueous solution.

2. The dormancy of Lovell peach seed was broken by treatment with thiourea.

3. Of the 22 other varieties similarly treated, the dormancy of six was not broken, ten germinated 10 per cent., and all remaining varieties responded to a lesser degree than did the Lovell.

4. Lovell seedlings which developed from non-after-ripened seed whose dormancy was broken by treatment with thiourea were dwarfed, with shortened internodes and anomalous leaves, typical of seedlings from non-after-ripened, excised embryos.

5. Complete after-ripening of peach seed accomplishes more than a conditioning of the seed to germinate; it also involves changes in the seed which result in vigorous seedlings free from dwarfish and anomalous characters.

6. The most favorable treatments to break the dormancy of non-after-ripened Lovell seed were soaking for 2 to 16 hours in 0.25 to 0.5 per cent. aqueous solution of thiourea or placing in a continuous supply of 0.25 per cent. solution.

7. Lovell seed which was treated with thiourea while still enclosed in the stony pericarp failed to germinate but germinated when removed from the stony pericarp.

8. Seed coats of after-ripened Lovell seed were checkered in appearance and the cotyledons became enlarged during germination, whereas non-after-ripened, thiourea-treated Lovell seed did not have this appearance and did not enlarge in this way.

9. Molds and common seed-borne fungi were reduced by treatment of seed with thiourea.

The authors are indebted to the following for providing seed used in these tests: DONLY GRAY, Elverta, Calif.; STANLEY JOHNSTON, South Haven, Mich.; HARRY MALTER, Greening Nursery Company, Monroe, Mich.; MALONEY BROTHERS, Dansville, New York; Maxwell-Bowden Nursery Company, Geneva, New York; Jackson & Perkins Company, Newark, New York; and especially DR. OMUND LILLELAND, and DR. E. L. PROEBSTING, University of California, Davis, Calif.

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PERMANENT WILTING PERCENTAGES OF SOILS OBTAINED FROM FIELD AND LABORATORY TRIALS

A. H. Hendrickson and F. J. Veihmeyer

(WITH FIFTEEN FIGURES)

In experimental work on the soil-water relations of plants in the field, it is advantageous to have some knowledge of the water-holding characteristics of the soil before the field trials are started. If the moisture equivalents or the field capacities and the permanent wilting percentages are known, an irrigation program for the various plots can be laid out in advance, with assurance that the unirrigated plots will not be allowed to remain dry too long and that the other plots will be watered according to plan. The difference between the field capacity and the permanent wilting percentage may be taken as the percentage of water readily available to plants. The moisture equivalent may be used as a measure of the field capacity of most medium- and fine-textured soils (**10**).

Since our experimental evidence has shown that the permanent wilting percentage is the critical soil moisture content denoting the lower limit of readily available moisture (**11**), the advantage of having accurate information concerning this constant is apparent. Sometimes, however, the permanent wilting percentages obtained in the laboratory or greenhouse, either by direct or indirect methods of determination, do not agree closely with the results obtained in the field. We believe that the permanent wilting percentages obtained directly in the field or in the laboratory are more satisfactory than those obtained by indirect methods. Calculation of the permanent wilting percentage from the moisture equivalent has proved to be notably untrustworthy in this respect (**8**). In certain cases field results also have not agreed closely with the permanent wilting percentages obtained by growing plants in containers, using a stock supply of soil previously obtained from the area on which the experiments were conducted, particularly when the soil was not uniform in texture or structure in the lower depths.

The fact that these discrepancies sometimes occur led to a re-examination of our methods of making permanent wilting percentage determinations in an effort to find out what factors were responsible, and, if possible, how the difficulties might be overcome. These studies led to a general realization that in most cases the direct determination of the permanent wilting percentage by growing plants in containers gave results in close agreement with those in the field; in others, satisfactory agreement could not be obtained.

Methods of finding the permanent wilting percentage

The direct determination of the permanent wilting percentage may be obtained by either of two general methods. The first method, which is

chiefly of value in regions of rainless summers, is by a system of regular soil sampling during the season. When the curves of the moisture contents of the soil samples are plotted, the percentage at which the curves become horizontal or nearly so, indicating the extraction of moisture has nearly ceased, may be taken as the permanent wilting percentage. While the permanent wilting percentage thus found is obtained under actual field conditions, is accurate and is reproducible in succeeding years, it has the disadvantage of being found comparatively late in the season. In order to determine whether the curve is horizontal or nearly so, several points are necessary. This means that the plants must be left wilted for at least several days, with the danger of some injury to the crop. Furthermore, it is difficult to plan intermediate irrigation treatments until the lower limit of readily available moisture is known. In effect, this means that one year must be devoted to finding the permanent wilting percentage before the experiment can be started.

The second method is by bringing into the laboratory samples of soil from the various depths, and by growing and wilting indicator plants on this soil. The soil not used for growing plants is saved as a stock supply on which other tests may be made later. This method offers several advantages—the work may be done during the winter months and the results obtained before the opening of the irrigation season. Furthermore, results may be secured from soils from widely separated areas, and tentative plans made to fit the various characteristics of the soils used. In some cases this procedure has a serious disadvantage. When the stock supply of soil is obtained from 6 to 8 places in the plot, we have found it convenient to use a fairly large post-hole soil auger. There is some mixing as the auger is turned down through the soil. The mixing presents no complications if the soil is uniform, but if layers of soil of widely different textures are encountered, the resulting sample is an indefinite mixture. Changes in texture are not easily discovered with this type of tool, as it is about as easy to bore through a clay as a loam, particularly when the soil is moist. Unfortunately, as the upper plane of the different textured subsoil is seldom parallel to the cultivated surface, the proportions of the two types of soil generally vary in each sample and also from those taken later in the season at different places in the plot.

Results

Comparison of field and laboratory trials

In certain cases the differences between the permanent wilting percentages obtained from sunflower plants in containers and those from the field were slight, while in others they were fairly large. In general, where there is a difference in results from plants in containers and in the field, the former are lower than the latter. On the other hand, Staples and Le Hane (5) reported results that indicate the soil-moisture content remaining when wheat plants in containers wilted, was higher than that found in the field

at harvest time. Further experience indicates that lack of agreement is generally due to lack of uniformity in the subsoil (**12**). The largest discrepancies are generally found in those cases where there is a sudden and rather wide change in texture which is reflected in a large increase in the moisture equivalent with increase in depth. Soils having fairly uniform moisture equivalents at the depths sampled, as a rule, show good agreement between the permanent wilting percentage obtained with sunflowers in containers and that observed in the field, when successive soil samples showed little or no extraction of moisture.

Part of this lack of agreement is probably due to the fact that the layers of soil of different texture do not occur at a uniform distance below the surface. In taking samples, whether randomized or according to a definite pattern, the operator secures samples that vary in the proportions of the different textured soils. For example, if we have a sandy loam overlying a silt loam, one core may consist of 75 per cent. sandy loam and 25 per cent. of the silt loam, while the next sample from the same depth may contain approximately half of each. The sample may also vary from the stock supply from which the permanent wilting percentages were obtained. The operator cannot always tell when the soil tube passes from a soil of one texture to that of another because of the slight change in the downward progress of the tube. An operator using a 30-pound hammer is usually unaware of slight differences in soil texture, especially when the soil is still moist. It does not seem practicable, therefore, to attempt to keep the various layers separate.

Soil samples from plots where there is a lack of adequate root distribution may vary because of the presence of a layer of soil that for some reason is not thoroughly permeated by roots. In a mature pear orchard reported previously (**12**), on soil of an average medium texture, layers of soil were found in which there was an abundance of roots while in others there was a scarcity. Samples taken through these layers showed an average moisture content slightly above the permanent wilting percentage, even late in the season when there had been ample time to extract the readily available moisture. It is probable that the inclusion of moist soil from the layers with a scarcity of roots raised the average of the sample. WORK and LEWIS (**15**), working with pears on a heavy clay soil, report a scarcity of fine roots. They advance the hypothesis for the soil with which they worked that even though the soil in contact with the roots may be dry, that, at a little distance away, may be moist. The sample taken with the soil tube includes portions of the dry and the wet soil, giving an average above the permanent wilting percentage.

Some of the results we have obtained are given in table I. The permanent wilting percentage of the first foot is lower in the field trials because of the inclusion of the surface mulch which lost moisture by direct evaporation. It is evident that the permanent wilting percentage obtained with sunflowers in containers, with few exceptions, agrees closely enough with

those obtained in the field below the first foot to be used in planning an irrigation program. Two exceptions occurred in the data given for grapes; one in the 3- to 4-foot depth of the San Joaquin series and in the 3- to 4-foot depth in the Yolo sandy loam. It will be seen in both these cases there was a marked increase in the moisture equivalent near the depth at which the lack of agreement occurred. In the case of the plums the lack of agreement was noticeable in the second and third foot depths, while a marked change in the moisture equivalents occurred in the third foot. The results obtained with peaches show agreement with those obtained with sunflowers.

In the pear orchard on the Yolo fine sandy loam, it was noticed that year

TABLE I

PERMANENT WILTING PERCENTAGES OBTAINED WITH SUNFLOWERS IN CONTAINERS AND FIELD TRIALS WITH OTHER CROPS

Soil	Depth of sampling	Moisture equivalent	P.W.P. obtained with sunflowers	P.W.P. Av. of field trials	Crop used in field trials
	ft.		%	%	
Fresno sandy loam	0–1	9.3	2.7	1.7*	Grapes
	1–2	8.3	2.6	2.6	"
	2–3	8.3	2.7	2.6	"
	3–4	8.2	2.7	2.8	"
	4–5	8.6	3.0	2.8	"
Fresno sandy loam	0–1	10.4	3.2	2.8	Grapes
	1–2	10.3	3.3	3.4	"
	2–3	10.7	3.4	3.4	"
	3–4	12.4	3.5	3.8	"
	4–5	13.9	3.5	4.1	"
San Joaquin loam	0–1	6.9	3.0	2.8	Grapes
	1–2	7.3	3.4	4.0	"
	2–3	7.6	3.7	4.1	"
	3–4	7.8	3.8	4.9	"
	4–5	10.2	5.0	5.3	"
Yolo sandy loam	0–1	17.0	8.3	6.3	Grapes
	1–2	16.3	8.6	9.2	"
	2–3	16.6	8.9	9.6	"
	3–4	20.7	9.8	11.2	"
	4–5	21.0	9.7	9.3	"
Yolo sandy loam	0–1	21.0	9.6	8.5	Grapes
	1–2	16.9	8.4	8.9	"
	2–3	14.1	7.3	7.7	"
	3–4	14.3	6.9	7.5	"
	4–5	13.3	6.3	6.6	"
	5–6	12.3	6.1	6.0	"
Fresno sandy loam	0–1	8.5	2.5	2.2	Peaches
	1–2	7.2	2.3	2.5	"
	2–3	7.3	2.4	2.5	"
	3–4	8.7	2.0	3.5	"
Madera clay loam	0–1	25.0	10.0	10.0	Peaches
	1–2	24.4	10.5	10.6	"
	2–3	23.7	11.0	11.0	"
	3–4	23.4	11.5	11.0	"
Yolo loam	0–3	22.2	10.8	9.5	Peaches
	3–6	17.7	9.1	8.7	"
	6–9	13.9	7.8	7.8	"

TABLE I—(*Continued*)

Soil	Depth of sampling	Moisture equivalent	P.W.P. obtained with sunflowers	P.W.P. Av. of field trials	Crop used in field trials
	ft.		%	%	
Yolo loam	0–1	24.2	12.5	11.6	Pears
	1–2	24.3	12.6	13.2	"
	2–3	22.7	11.8	12.3	"
	3–4	19.6	10.5	11.0	"
	4–5	21.6	10.3	10.5	"
Arvin loamy fine sand	0–1	8.6	4.2	4.2	Plums
	1–2	9.9	4.9	6.2	"
	2–3	12.3	5.3	6.7	"
	3–4	13.1	6.3	6.5	"
	4–5	9.4	5.1	4.6	"
	5–6	8.8	4.1	3.9	"
	6–9	12.1	5.0	4.8	"
	9–12	15.7	6.5	6.5	"
Yolo sandy loam	0–3	21.4	9.8	8.9	Prunes
	3–6	15.1	7.8	8.2	"
	6–9	17.8	10.6	10.3	"
Yolo loam	0–1	23.9	12.6	11.5	Lima beans
	1–2	22.2	12.5	12.5	" "
	2–3	21.7	12.5	11.9	" "
Yolo loam	0–1	22.6	10.8	8.5	Sugar beets
	1–2	21.0	9.9	10.2	" "
	2–3	19.0	9.0	9.0	" "
	3–4	16.6	8.1	8.3	" "
	4–5	15.2	8.0	7.6	" "
	5–6	14.9	7.5	7.6	" "
Yolo silt loam	0–1	23.2	11.1	10.3	Tomatoes
	1–2	23.3	11.0	10.8	"
	2–3	19.8	9.4	10.2	"
	3–4	17.6	8.6	9.6	"
	4–5	15.6	8.2	8.4	"
	5–6	15.3	7.7	8.4	"
Panoche adobe	0–1	35.5	16.0	15.5	Cotton
	1–2	31.7	16.0	16.0	"
	2–3	29.9	17.5	18.0	"
	3–4	29.9	19.0	19.5	"
Gleason loam	0–1	28.7	12.1	11.0	Manzanita
	1–2	29.1	12.8	12.3	"
	2–3	28.9	12.7	12.7	"
Aiken loam	0–1	26.4	10.2	9.7	Manzanita and scrub oak
	1–2	25.6	12.1	12.5	" "
	2–3	25.1	12.0	12.0	" "

* Includes soil dried by evaporation from the surface.

after year there was lack of agreement between the permanent wilting percentages obtained in the field and those in the laboratory in the second and third foot depths. Agreement was satisfactory at other depths. The soil samples from the second and third feet, even late in the season when the fruit had markedly slowed down in growth, showed a higher percentage of moisture than the permanent wilting percentage obtained by growing sunflower plants. A trench was dug near one of the experimental trees. Samples taken from the wall of the trench showed that there was an irregu-

iar layer of fine-textured material between the 2- and 4-foot depths. The different proportions of fine- and coarse-textured soils obtained in successive samplings helped to account for the irregularities in the soil-moisture curves for these depths. The moisture equivalents, indicating the variability of the soil encountered, are given in figure 1.

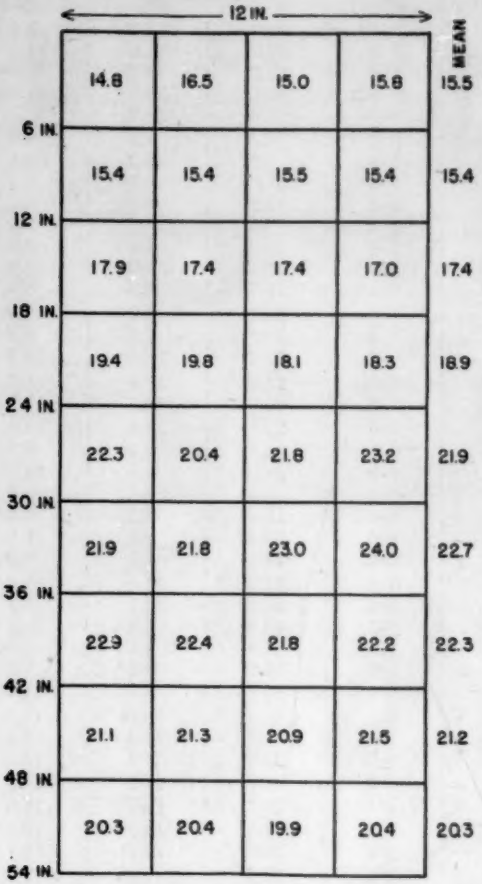

FIG. 1. Moisture equivalents of the top 4½ feet of Yolo fine sandy loam in a pear orchard showing marked but somewhat irregular changes beginning at about the 2-foot depth.

Another case is where uniform surface soil is underlain with pockets of coarse sand or fine gravel. Figure 2 shows wide variation in texture encountered in closely spaced samples. Areas of this type are frequently found in relatively narrow valleys. The results from soil-sampling under these conditions are often hard to interpret because samples taken late in the season sometimes have a higher moisture content than those taken earlier, probably due to the fortuitous inclusion of a large proportion of fine-textured material of comparatively high water-holding capacity. Irregu-

larities in the soil-moisture curves make it difficult to plot the point on the time axis when the extraction curve showed marked change in direction because of the exhaustion of readily available moisture. Wherever these discontinuities are present there is always the question of root distribution.

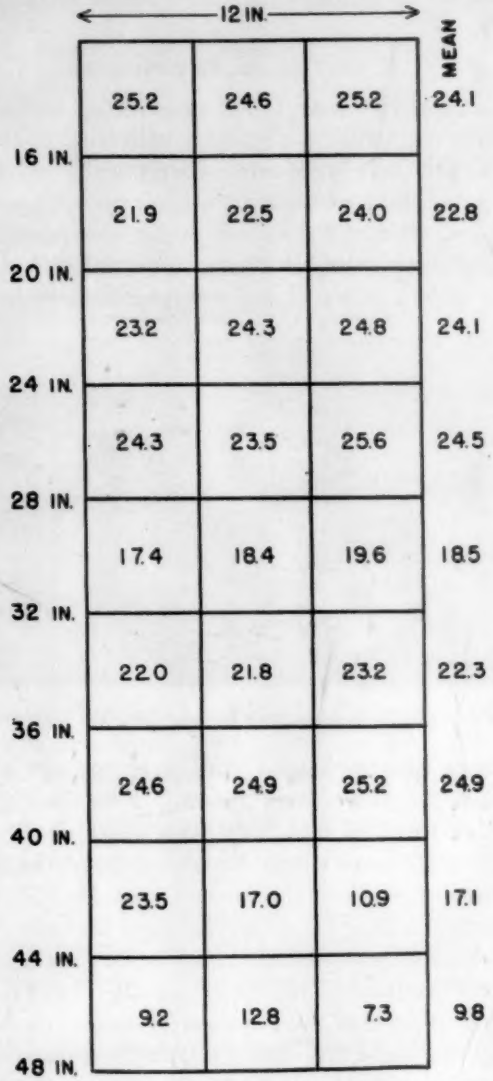

FIG. 2. Moisture equivalents of the 12- to 48-inch depth of Yolo silt loam, stream-bottom phase, showing marked changes in texture due to the presence of sand pockets.

In still another case (12) we found by digging a trench close to a mature pear tree that there were layers of soil differing in texture, and that some of these layers contained an abundance of roots while in others there were but few. The moisture content of the layer in which there was a scarcity of

roots remained at a high level for a longer time than that in the layers in which there was an abundance. It is interesting to note that gravel, if uniformly distributed, does not necessarily interfere with obtaining a consistent soil-moisture record, and one in which there is close agreement between the permanent wilting percentages obtained in the laboratory and in the field (**13**).

Effect of size of container

While in most cases we have found satisfactory agreement between the permanent wilting percentages obtained with sunflowers and with field results, the fact that there were some discrepancies led to a study of the technique used in obtaining permanent wilting percentages using sunflowers in small containers. Some of these containers are shown in figure 3. Our experience during many years has shown that sunflowers are well suited for use as indicator plants. One of the first problems to be studied was the

Fig. 3. Sunflower plants in containers holding 100, 250, and 600 grams of soil.

possible effect upon the permanent wilting percentage of the size of the container in which the plants were grown. Five sizes of containers were used, those holding 100, 250, 600, 3,350, and 9,600 gm. of dry soil. It was thought that the small cans would be advantageous in those cases where only small amounts of particular soils were available. Repeated trials gave the results presented in table II. The data show that the permanent wilting percentages obtained were essentially equal in all sizes used.

The small-sized containers had to be watched carefully and placed in the moist chamber as soon as the plants were judged to be wilted. If left exposed too long, the slight loss through transpiration after wilting constituted a considerable portion of water held in the comparatively small amount of soil used. We have found that number 2 friction-top tin cans, holding about 600 gm. of soil, are very satisfactory.

Effect of certain temperatures

Comparisons were next made under different temperature conditions. These afforded a comparison between results obtained under the fluctuating

TABLE II

PERMANENT WILTING PERCENTAGES OBTAINED BY GROWING SUNFLOWERS IN DIFFERENT SIZED CONTAINERS

Size of container	Yolo fine sandy loam		Yolo silty loam		Yolo clay	
	Number of trials	Permanent wilting percentage	Number of trials	Permanent wilting percentage	Number of trials	Permanent wilting percentage
gm.		%		%		%
100	62	8.6 ± 0.04†	71	11.9 ± 0.04	.....	.....
250	74	8.7 ± 0.03	71	12.0 ± 0.05*	.....	.....
600	60	8.6 ± 0.03	44	11.8 ± 0.03*	52	14.5 ± 0.03
3,350	.....	.....	.....	.....	36	14.4 ± 0.03
9,600	.....	.....	.....	.....	12	14.5 ± 0.06

* The difference between 12.0 and 11.8 is significant by odds of 44.9 to 1.
† Probable error. Differences are considered significant with odds of about 30 to 1.

conditions that ordinarily prevail in a greenhouse and under conditions where the plants were grown under uniform light and temperatures that did not vary more than two or three degrees. The results are given in table III.

Two series of plants were grown on two soils under constant temperatures with light intensities of about 500 foot-candles for 12 hours each day. The temperatures used were 55° and 75° F. The wilting percentages obtained, when both tops and roots were kept at these temperatures, were essentially equal for each soil. In another pair of experiments the roots were kept at 55° and at 41° F. by keeping the containers nearly immersed in a water bath, while the tops were exposed under constant illumination to a variable room temperature that averaged 70° F. In the series with roots at 55° F. the wilting percentage showed close agreement with the results obtained in the first two series. When the roots were kept at 41° F., however, the

TABLE III

PERMANENT WILTING PERCENTAGES OBTAINED BY GROWING SUNFLOWERS AT DIFFERENT TEMPERATURES

Yolo fine sandy loam		Yolo silty loam		Temperatures
Number of trials	Permanent wilting percentage	Number of trials	Permanent wilting percentage	
	%		%	°F.
90	8.6 ± 0.03	76	12.0 ± 0.03	Tops and roots at 55°
88	8.6 ± 0.03	54	11.9 ± 0.05	Tops and roots at 75°
50	8.5 ± 0.03	.....	.....	Tops at room temp., roots at 55°
34	9.0 ± 0.11	.....	.....	Tops at room temp., roots at 41°
18	8.6 ± 0.07	.....	.....	Cans protected with opaque covers
20	8.7 ± 0.04	.....	.....	Plants protected with cloth shade
38	8.7 ± 0.05	31	12.1 ± 0.06	Whitewashed greenhouse
48	7.8 ± 0.04	47	10.9 ± 0.06	Metal containers exposed to direct sunlight in greenhouse

wilting percentage showed an increase above those obtained at higher temperatures. These results are in accord with those reported by WHITFIELD (14) and CLEMENTS and MARTIN (3). In the remaining series where the plants were grown under the variable temperatures of a heated greenhouse, the wilting percentages obtained showed close agreement with those under the controlled temperatures of 55° and 75° F., except when direct sunlight reached the metal containers in an inadequately whitewashed greenhouse. The results obtained in the latter case were lower than those in which the metal containers were kept at room temperature.

When the cans were exposed to the direct sunlight, temperatures over 130° F. were observed in the center of the soil mass, and it is probable that there was some loss of moisture through the cotton seal around the stem of the plant. The permanent wilting percentages obtained were not affected

FIG. 4. Boxes used to protect metal containers from direct rays of the sun.

by the different air temperatures but were changed when the soil was kept either at 41° F. or allowed to reach abnormally high soil temperatures when exposed to direct sunlight. The minimum and maximum soil temperatures reported are beyond those likely to be encountered during the growing season in the field. Protection from direct sunlight on containers may be obtained by growing plants under cheesecloth shelter or in boxes similar to those shown in figure 4.

USE OF WATER BY PLANTS AFTER WILTING

Plants, whether grown in the field or in containers, continue to use some soil moisture even after they are wilted. After wilting, the loss of moisture by plants in containers continues at a faster rate than by plants in the field, as discussed later. Figures 5, 6, and 8 show the extraction of water by sunflowers from a Yolo clay, when grown in three sizes of containers. The accompanying photographs, figures 7 and 9, show the conditions of the plants at the moisture contents indicated on the curves. It will be noted that the first part of the curve (fig. 5) shows rapid extraction of moisture by the plant. As shown in the photographs there is no difference in appear-

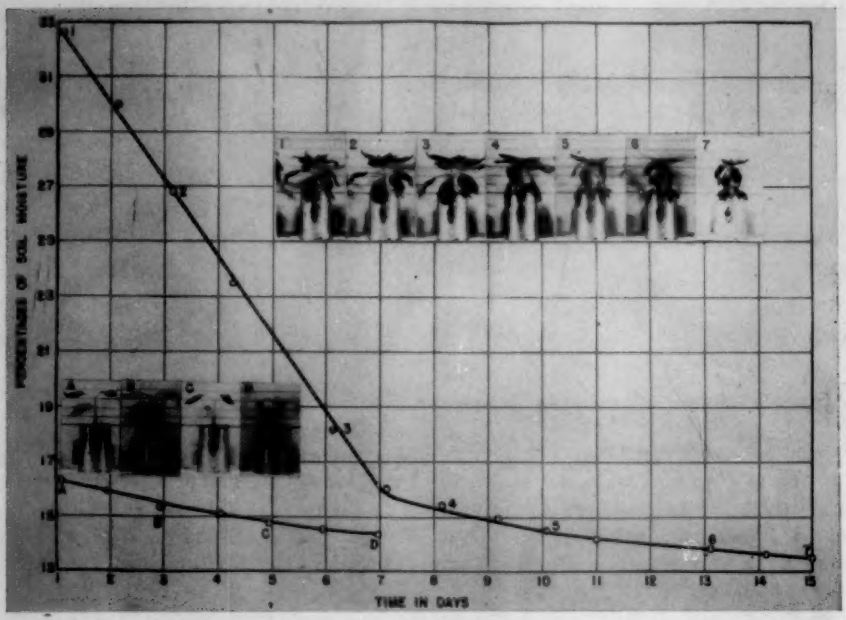

FIG. 5. Soil-moisture extraction curves obtained with a sunflower plant used first as an entire, and then as a two-leaved plant on 600 grams of Yolo clay. The number and letters on the photographs correspond to those on the respective extraction curves. The unpruned plant began to wilt between the 7th and 8th days (no. 4) and was permanently wilted when the moisture content was reduced to 14.5 per cent. (no. 5). The two-leaved plant wilted permanently at 14.4 per cent. (D).

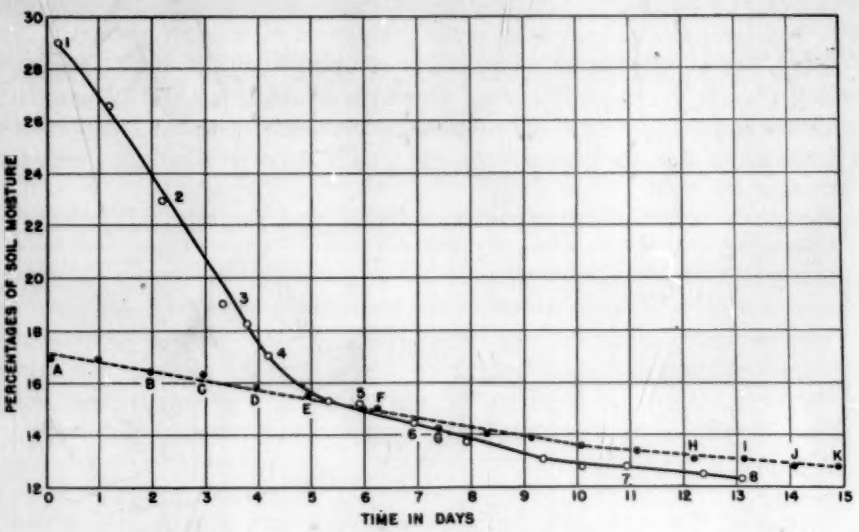

FIG. 6. Soil-moisture extraction curves obtained with a sunflower plant, first as an entire and then as a one-leaved plant on 3,350 grams of Yolo clay.

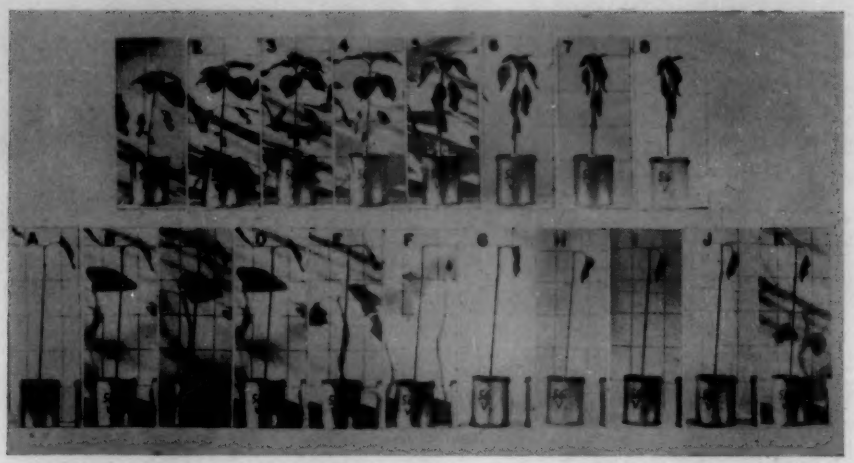

FIG. 7. The sunflower plant used to obtain the curves in figure 6. The numbers and letters on the photographs correspond to those on the respective extraction curves. The unpruned plant, solid line, began to wilt at 15.2 per cent. (no. 5) and was permanently wilted at 14.5 per cent. (no. 6). When reduced to one leaf, dotted line, it wilted permanently at 14.2 per cent. (G).

ance of the plant when the moisture dropped from 32.6 to 16 per cent., but a decided change in appearance occurred during the next day when the moisture dropped to 15.4 per cent. However, it was not until the moisture had reached 14.5 per cent. that the plant was judged to be permanently wilted. Experience with similar plants led us to believe that the plant at 15.4 per cent. soil moisture would have revived if placed in a moist chamber. We therefore took 14.5 per cent. as the permanent wilting percentage.

In a similar experiment using gallon containers with about 3,350 grams of soil (figs. 6, 7), the entire plant showed some drooping at 15.2 per cent. moisture and the plant was judged to be permanently wilted at 14.5 per cent. (shown as no. 6 in the photographs and on the curve). Similarly, with the

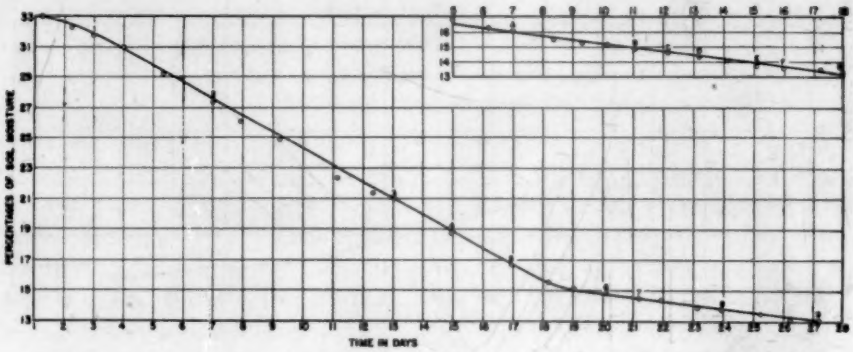

FIG. 8. Soil-moisture extraction curves obtained with a sunflower plant used first as an entire plant, and then as a two-leaved plant (upper right) on 9,600 grams of Yolo clay.

one-leaved plant, when the moisture content reached 14.2 per cent. (G), the plant was judged wilted.

In the third experiment, with sunflowers in pails holding about 9,600 gm. of soil (figs. 8, 9) some drooping occurred when the soil moisture reached 14.8 per cent. and the plant was judged permanently wilted at 14.5 per cent. (no. 7). With the two-leaved plants the first drooping occurred at 14.9 per cent. and permanently wilted at 14.4 (D).

It is clear from the photographs that there is a comparatively narrow range of soil-moisture content in which the appearance of the plant changes appreciably. In this part of the curve, the plant will revive if placed in the moist chamber; will wilt fairly quickly when taken out of the moist

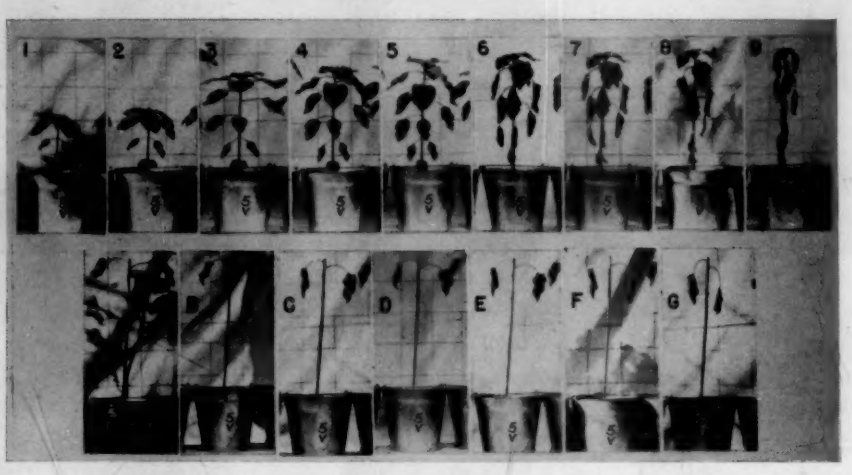

FIG. 9. The sunflower plant used to obtain the curves in figure 8. The numbers and letters on the photographs correspond to those on the respective extraction curves. The unpruned plant began to wilt at 14.8 per cent. (no. 6) and wilted permanently at 14.5 per cent. (no. 7). When reduced to two leaves it wilted permanently at 14.4 per cent. (D).

chamber; and will undergo successive wiltings and revivals, until it finally reaches a condition of wilt from which it will not revive.

We have pointed out (9) that this succession of soil moisture percentages represents a range in which the plant passes from full turgidity to permanent wilting. Obviously, there is some leeway in judging when a plant is permanently wilted since it is impossible to tell when a plant has gone beyond the permanent wilting stage. Therefore, the permanent wilting percentage represents a narrow range of moisture contents rather than a unique value. Generally, this value may be obtained from plants in containers within about one per cent. with the fine-textured soils and about 0.5 per cent. with the sands. For example, in figure 5 it will be seen that in the 600-gram containers there is a drop of 0.9 per cent. in soil-moisture content from the time drooping became apparent to that of permanent wilting; in figure 6 the corresponding drop was 0.7 per cent.; while in figure 8, it was 0.3 per cent. A further example is shown in figures 10 and 11 in

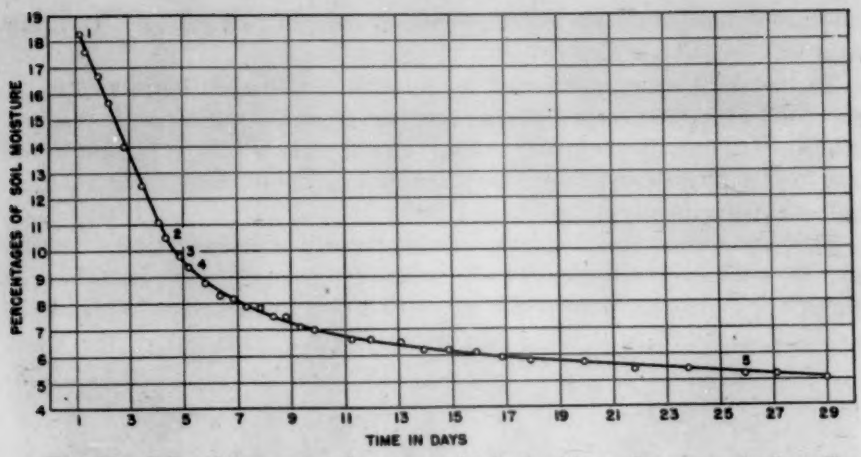

FIG. 10. Soil-moisture extraction curve obtained with sunflower, mustard, mallow, and sorghum plants on 9,600 grams of Madera silt loam.

which the soil-moisture extraction curve obtained from sunflower, mustard, mallow, and sorghum plants on Madera silt loam show that there is a drop of only 0.4 per cent. from visible drooping to permanent wilting. The narrow

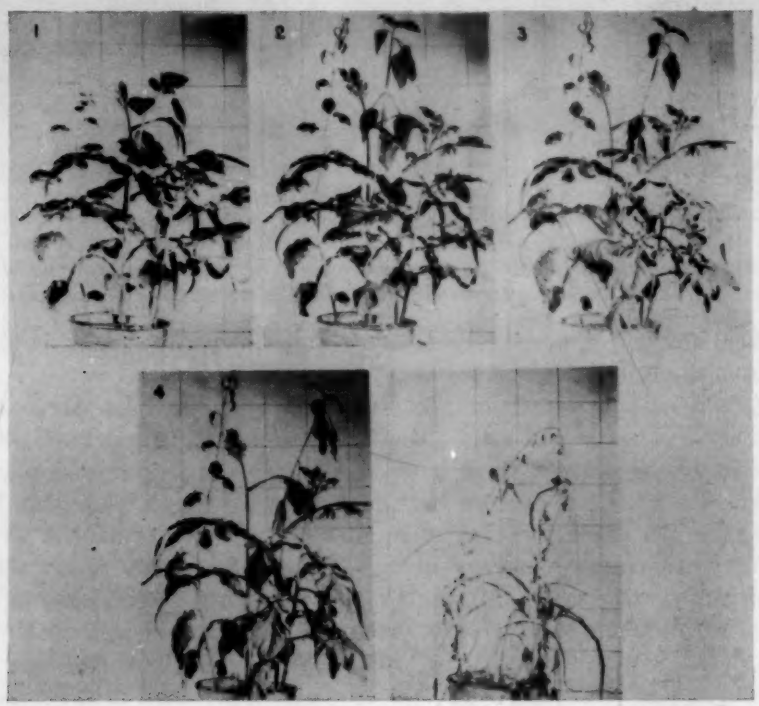

FIG. 11. Plants used to obtain the curve in figure 10. The numbers on the photopraphs correspond to those on the curve. The plants began to wilt at 9.8 per cent. (no. 3) and were permanently wilted at 9.4 per cent. (no. 4).

range of soil-moisture contents between the first evidence of drooping and permanent wilting is illustrated by the plants in figure 12. These sunflower plants were grown in small containers on Fresno sand. The difference in soil-moisture contents between the plant showing droop and the one permanently wilted was 0.3 per cent.

It is well known that all of the leaves on a plant do not wilt at the same time and the question then arises as to what part or parts of the plant should be used as a criterion of wilting. Should the whole plant be allowed to wilt, or should the bottom leaves or some particular pair of leaves be used? In this connection it should be pointed out that it is impossible to tell whether

FIG. 12. These 6 sunflower plants on Fresno sand, with the same amount of soil, illustrate the narrow range of soil-moisture contents within which wilting takes place. The gross weights of the cans and the soil-moisture percentages are:

Can no.	97	105	101	94	102	104
Gross wt. (gm.)	841.4	843.1	843.9	845.3	847.0	847.3
Moisture (%)	3.3	3.6	3.7	3.9	4.1	4.1

Plants 104 and 102 are turgid. Number 94 shows a slight drooping of first leaves; number 101 is in a more advanced stage of wilting; number 105 is permanently wilted; and number 97 has passed this stage.

a plant has gone beyond permanent wilting. If it has not reached the permanent wilting percentage, however, recovery takes place in the moist chamber, and it may again be placed under the former evaporating conditions. The plant which has just permanently wilted is essentially no different in appearance from one which has been wilted several hours. The use of several of the latter in computing the permanent wilting percentage for a series tends to make the average too low. On the other hand, plants which show recovery in the moist chamber are not used in the computations and as a result the average permanent wilting percentage is seldom too high. Permanent wilting percentages obtained from plants in containers may be too low instead of too high because we have a satisfactory test or check in plants that are *not* wilted, but we have no check on plants that have gone beyond wilting.

Figures 5, 6, 8, and 10 show that reduction of soil moisture with plants in containers continues at a fairly rapid rate even after the plants have wilted. It may continue up to, or even after, the death of the plant. This is in substantial agreement with the work of TAYLOR, BLANEY, and MCLAUGHLIN (7) who show similar results. They have proposed the term "ultimate wilting point" to designate the soil moisture content when all the leaves have wilted, and define the wilting range as "the range in moisture-content of the soil between the wilting coefficient and the ultimate wilting point."

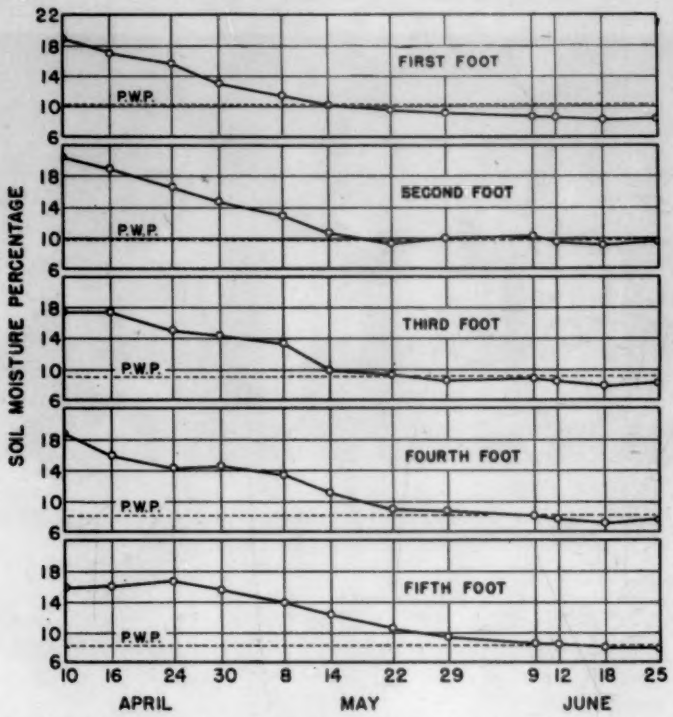

FIG. 13. Soil-moisture extraction curves obtained with sugar beets on Yolo loam. The permanent wilting percentages obtained with sunflowers in small containers are indicated by the broken lines.

The reduction below the permanent wilting percentage obtained in the field, in our experience, is much less than that from plants in containers. For example, with sunflowers in small containers (fig. 5) the loss after the first sign of wilting was 1.9 per cent. in 7 days; from another series of large containers the loss was 1.7 per cent. in 7 days, and from a third (fig. 10) 4.7 per cent. in 24 days, at which time the plants appeared dead. In contrast, in the field, the loss of soil moisture below the surface layer from the time the moisture curves became horizontal or nearly so was one per cent. or less during the remainder of the growing season, which may be 3 or 4

months, as shown in figures 13, 14, and 15. Similar results were obtained with walnuts (**12**). The reasons why plants in containers reduce the soil moisture below that found in the field after wilting are not known, but the loss seems to be greater than could be attributed to that through the seal. If plants in containers are allowed to reduce the moisture below permanent wilting percentage, the selected percentage thus obtained is lower than that obtained in the field. While the differences in moisture contents between the permanent wilting percentage and "ultimate wilting point" obtained from plants in containers may be comparatively large, the differences be-

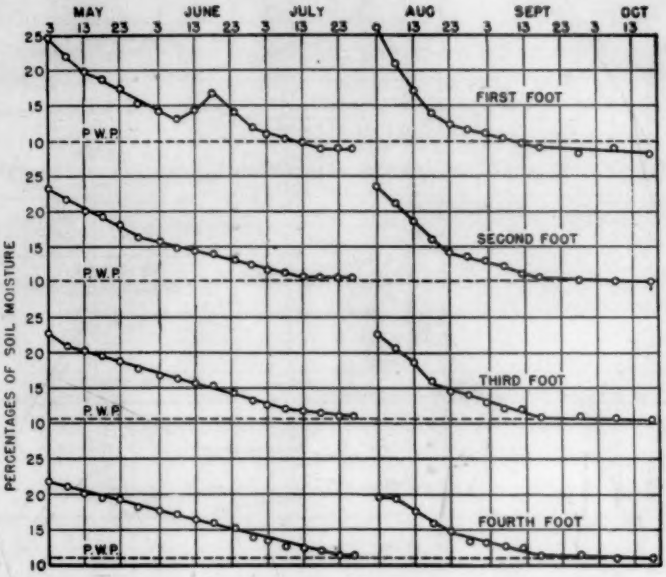

FIG. 14. Soil-moisture extraction curves obtained with peach trees on Madera clay loam. The permanent wilting percentages, obtained with sunflowers in small containers, are indicated by the broken lines. The increase in soil moisture in June was due to rain, and that early in August, to irrigation.

tween the permanent wilting percentage and the minimum moisture content under field conditions are so small as to be unimportant.

When a fruit tree reduces the moisture to the permanent wilting percentage (as shown by the horizontal flattening of the curve) not all of the leaves appear wilted. The fruit, however, if present, shows a marked slackening in growth. It does not seem logical to assume that with sunflower plants we must wait until all of the leaves droop before we consider the plant wilted. We have found that with sunflowers the use of the second, third, and fourth pairs of leaves gives results that agree closely with the values obtained in the field, except in cases similar to some of the conditions pointed out in connection with table I. The leaves used, of course, must be uninjured and free from insects and diseases.

Effect of removal of all but one or two leaves

Indecision as to what leaves to watch for wilting may be avoided by reducing the plant to one leaf or a pair of leaves as illustrated in figures 3 and 7. By using 1- or 2-leaved plants the "wilting range" may be materially reduced; when the leaves will not revive in a moist chamber, there are no others present to influence the judgment of the operator. The permanent wilting percentage was determined for a large number of plants

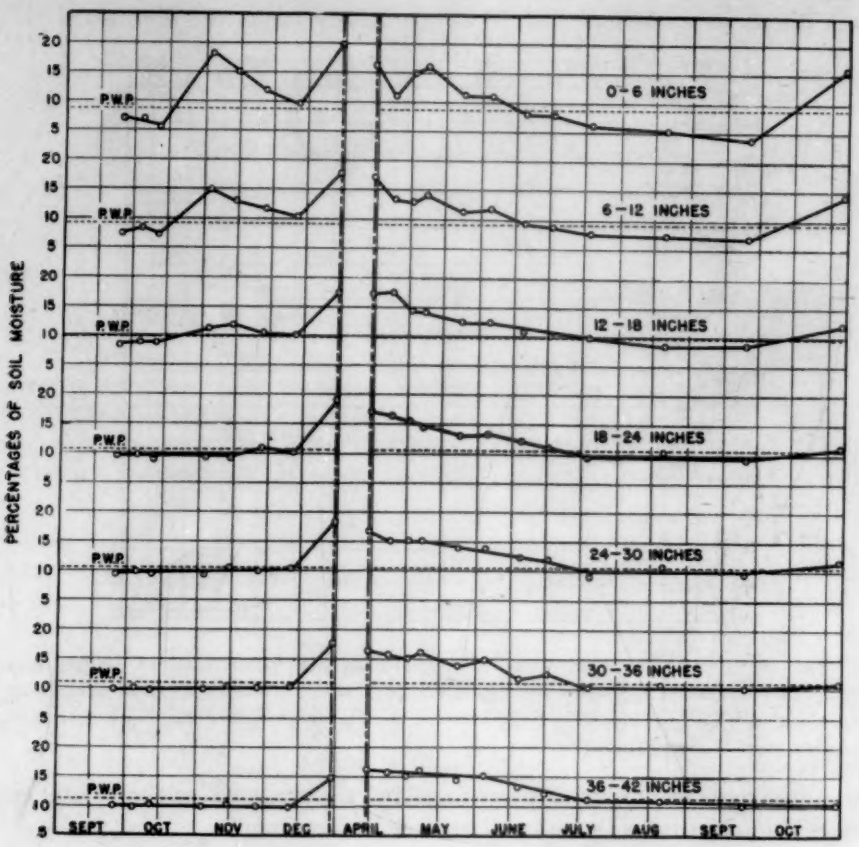

FIG. 15. Soil-moisture extraction curves obtained with native sclerophyllus vegetation on Hugo loam. The permanent wilting percentages, obtained with sunflowers in small containers are indicated by the horizontal dashed lines. The increases in soil moisture are due to winter rains. A portion of the record during the winter months is omitted.

that had been reduced to one or two leaves on each plant. The lower leaves and cotyledons were removed. The stem was then cut just above the test leaves. Large numbers of both the entire and the 1- and 2-leaved plants were allowed to wilt under the various evaporating conditions described earlier in this report, with the results given in table IV. No differences were obtained whether entire plants or 1- and 2-leaved plants were used. Sub-

stantially similar results obtained with individual plants are shown, also indicated in figures 5, 7, and 9.

CONSTANCY AND RECURRENCE OF THE PERMANENT WILTING PERCENTAGE IN THE FIELD

The curves from the natural vegetation (fig. 15) and from the peach orchard (fig. 14) illustrate another important fact: i.e., that the reduction of soil moisture to essentially the same percentage occurs repeatedly. This condition occurs annually in areas of native vegetation, or on unirrigated crop land, and may be brought about in irrigated sections by withholding water. Under semi-arid conditions the soil is reduced to the permanent wilting percentage at some time during the growing season (in many places by mid-July). It then generally continues to lose moisture slowly (but

TABLE IV

PERMANENT WILTING PERCENTAGES OF ENTIRE SUNFLOWER PLANTS AND ONE- AND TWO-LEAVED PLANTS

PLANT MATERIAL	YOLO FINE SANDY LOAM		YOLO CLAY	
	NUMBER OF TRIALS	PERMANENT WILTING PERCENTAGE	NUMBER OF TRIALS	PERMANENT WILTING PERCENTAGE
		%		%
Entire plant	50	8.7 ± 0.03	86	14.4 ± 0.02
One- and two-leaved plants	56	8.7 ± 0.04	14	14.4 ± 0.05

usually not more than about one per cent. for the remainder of the season) until moistened by winter rains. Data reported by TAYLOR, BLANEY, and MCLAUGHLIN (7) show similar results for the second, third, and fourth feet of soil in their field plot.

In terms of volumetric water content such as acre inches the differences between the field capacity and the permanent wilting percentage is slightly less than the amount of water that must be added to the soil each fall before any water drains away. To this must be added the additional water lost by direct evaporation from the surface layers. Inasmuch as the permanent wilting percentage of a soil in a given area is a constant, the amount of water required each fall before there is any addition to the underground supply is also essentially constant, if there is no change in the type of vegetation.

Discussion

Soil-moisture extraction curves (figs. 13, 14, 15) plotted from results with plants under field conditions, slope downward approximately uniformly until they reach a moisture content at which there is a marked reduction in rate of extraction. This change in direction of the curve occurs slightly above the permanent wilting percentage obtained from sunflower plants in containers. After reaching this moisture content the curves, from results

in the field, continue nearly horizontal, showing that the soil moisture is slowly reduced to a minimum which is slightly below the permanent wilting percentage. This reduction is small, often not more than one per cent., even after 3 or 4 months. These extraction curves practically coincide, year after year, both as to slope and as to the minimum moisture content reached, if the vegetation is not changed.

BRIGGS and SHANTZ (1) showed the nearly horizontal curves that are found after the plants have wilted. BURR (2) also showed that with wheat the soil-moisture extraction curves were substantially horizontal in the latter part of the growing season. On the contrary, TAYLOR and FURR (6) presented a soil-moisture extraction curve for citrus from which they concluded there was a downward slope approximately the same as that obtained from sunflowers in containers.

ESSELEN (4), however, also working with citrus fruits, showed that the extraction curves in the unirrigated portions of the orchard were horizontal, or nearly so, for about 6 months.

The extraction curves resulting from growing of entire plants in small containers, on the other hand, slope downward rather steeply after the plants are permanently wilted. The reasons for the downward slope of the curves from plants in containers are not all apparent. It is known, however, that plants continue to use some water after they are wilted, although not at as rapid a rate as before; with the limited amount of soil in the containers, even a slight extraction by the wilted plants may make an appreciable difference in the percentage of moisture present. We find that the minimum moisture content in containers that may be obtained by waiting a considerable period after the plants have wilted, is much lower than that found after a similar, or even much longer delay, in the field.

While an error may be made in the field because it is difficult to tell when some plants, particularly trees and certain native plants, are wilted, nevertheless, a delay of a few days after wilting under field conditions does not give a value much below the permanent wilting percentage. On the contrary, because of the relatively steep extraction curve of plants in containers after wilting and the delay of a few days possibly due to error in judgment, the value found may be considerably below the permanent wilting percentage. With careful work, however, agreement between results with plants in containers and those in the field may be obtained.

Another discrepancy between field and laboratory results is attributed to the presence of layers or pockets of soil of widely different textures. Under these conditions, close agreement between field and laboratory results is sometimes unobtainable. Still another discrepancy is caused by the lack of adequate root distribution, as has been reported from results with pear trees (12, 15), and which may also occur with annual plants before the root system is fully developed. Neither of these discrepancies is due to errors in judgment on the part of the investigator.

The question then arises as to what criterion to use to judge whether a

plant is wilted or not. The first signs of wilting are a loss of turgor by the lowest leaves, accompanied by a slight reduction in the rate of loss which may be detected by frequent weighings. If placed in the moist chamber, these plants revive. When placed in the light, the drooping again occurs and is soon noticeable in other leaves above those in which the wilt was first seen. Finally, the leaves do not revive, and we call the resulting moisture content of the soil the permanent wilting percentage. When plotted on a large scale (figs. 5, 6, 8, 10), it will be found that the permanent wilting percentage is at a moisture content slightly below the first reduction in rate of loss that occurred when the leaves first began to droop. The fact that it is so easy to allow the plant to remain wilted too long, and that slight errors in judgment are bound to occur, shows that it is practically impossible to assign a unique value to the permanent wilting percentage, and that this soil moisture condition must cover a small range of moisture contents. In clay soils this range need not exceed 1 per cent., and in sands, more than 0.5 per cent. If all of the leaves on a plant in a container are allowed to wilt to ascertain the permanent wilting percentage, the resulting value is much lower than that found under field conditions. Furthermore, with sunflowers, when all the leaves have wilted, some of them are dead. The minimum moisture content obtained with plants in containers, when all the leaves are allowed to wilt, and which corresponds to the "ultimate wilting point" is of questionable value in irrigation practice because it is a moisture content lower than that reached in one season on irrigated soils, except in the surface layer where direct evaporation is a factor.

Losses from the relatively small container would doubtless continue whether the plant is dead or alive, until finally the soil reaches the air-dry condition. If the "ultimate wilting point" is defined as the moisture content at which all the leaves wilt, confusion can be eliminated by using a plant with but one leaf. The permanent wilting percentage and the ultimate wilting point would then coincide.

We have only one visual criterion for wilting in sunflower plants, and that is whether the plant revives or not when placed in the moist chamber. If the selected leaves revive, the plant is temporarily wilted; if they do not, it is permanently wilted. Unfortunately, this criterion gives no indication if the selected leaves have gone beyond wilting. We have not been able to pick out intermediate stages of wilting such as "first permanent wilting" with a sufficient degree of accuracy to make these values useful.

The constancy of the permanent wilting percentage and its recurrence within close limits each season in regions of rainless summers, as illustrated in the results obtained with native vegetation, makes it a useful base from which to calculate the amount of rainfall needed to replenish the water used by plants. In general, the total rainfall minus the sum of the amount needed for this replenishment, plus the amount lost by direct evaporation, plus the runoff, gives the rainfall that finds its way to the underground supply. Similarly, the amount of water required in irrigation practice to

replenish the moisture readily available to plants may be calculated if the permanent wilting percentage is known.

Summary

1. Agreement in the permanent wilting percentage from plants in containers and in the field can be obtained when the soil is uniform or nearly so.

2. Lack of agreement occurs when marked irregularities are found in the field in the soil occupied by roots.

3. The permanent wilting percentages obtained with sunflower plants agreed whether the determinations were made on entire plants or plants with either a single leaf or a pair of leaves.

4. The permanent wilting percentages agreed whether large or small containers were used.

5. Permanent wilting percentages obtained in these experiments were not affected by temperature conditions studied in these experiments except in those cases where the soil was kept at 41° F. and when the soil was raised to a relatively high temperature by direct sunlight on the metal containers.

6. The minimum soil moisture content found with plants in containers was lower than that found under field conditions, except in the top layer.

7. The permanent wilting percentages is a recurring soil-moisture condition. Native vegetation or crops under dry farm conditions reduce the soil-moisture content to about the same percentage year after year. Irrigated crops do the same thing if the intervals between irrigations are long enough.

8. The permanent wilting percentage, as we define it, is not a unique value but is a small range of soil-moisture contents within which permanent wilting takes place. This range need not exceed one per cent. for fine-textured soils or 0.5 per cent. for coarse-textured ones.

We are indebted to Dr. L. D. Doneen for some of the data in table I and for figure 13.

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THE INFLUENCE OF MINERAL NUTRITION, SOIL FERTILITY, AND CLIMATE ON CAROTENE AND ASCORBIC ACID CONTENT OF TURNIP GREENS

LEON BERNSTEIN, K. C. HAMNER, AND R. Q. PARKS

(WITH FOUR FIGURES)

Previous investigations from this laboratory with tomatoes (**2, 7**) have indicated that the ascorbic acid and carotene content of the tomato fruit is influenced but little by the supply of mineral nutrients to the plants. This work was done with a fruit, however, and it was thought that in a leafy crop like turnip greens the influence of mineral nutrient supply and soil fertility upon these two constituents might be greater than in the tomato. The two experiments reported here are concerned with the effect of the supply of nutrient elements in sand and in soil cultures on the vitamin content of turnip greens. Subsequent experiments to be reported elsewhere will deal with the effects of other environmental factors. In all experiments the Shogoin variety of turnips was used.

Several investigators (**11, 14, 16**) have noted that the content of several vitamins and minerals is relatively high in turnip greens. This particular investigation deals with the ascorbic acid and carotene contents.

KELLER and MINOT (**9**) report that the ascorbic acid content of fresh turnip greens varied from 75–160 mg. per 100 gm. fresh weight (with an average of 115 mg.) in ten samples. TUL'SCHINSKAYA (**18**) found in each 100 grams of fresh leaves 51.9 mg. of ascorbic acid in July, 76.7 mg. in August, and 105 mg. in September. FLOYD and FRAPS (**5**) state that the greens vary from 67 to 422 mg. of ascorbic acid per 100 gm. fresh weight. REDER, ASCHAM, and EHEART (**15**) found variations from 116 to 257 mg. per 100 gm. fresh weight. They grew turnips in well-replicated fertilizer experiments at four locations in the south. In their summary they state: "Wide variations were obtained in the ascorbic acid content of greens produced at the four places; the mean ascorbic acid content of greens at Norfolk, Virginia (2.4103 mg. per gm.), was nearly twice that of greens at Blacksburg (1.2842 mg. per gm.). In the four experiments, the influence of place was 13.75 times as great as the most important average effect produced by fertilizer treatment within a single locality. These variations did not appear to be directly related to differences in soil composition or to differences in temperature. . . . These results seem to indicate that the formation of ascorbic acid may be influenced by light intensity and rainfall as well as by fertilizer applications."

Methods of analysis

ASCORBIC ACID

The method of preparing the extracts has been described by MORELL (**13**). The extract of the sample after grinding was filtered through Whatman

no. 2 paper and an aliquot of the clear, colorless filtrate titrated to an end-point with standardized 2,6-dichlorophenol-indophenol dye. Each liter of the acid used for the extraction contained 20 gm. of metaphosphoric acid and 20 ml. of concentrated sulfuric acid. The titration method was checked by comparison with the method of BESSEY (1) and found to agree.

DEHYDROASCORBIC ACID

The method employed was a slight modification of the method of GUNSALUS and HAND (6). An extract was prepared from 10–25 gm. of fresh turnip leaves in a Waring Blendor with 100 ml. of a 1 to 10 dilution of the acid mixture used for extraction of ascorbic acid; i.e., a mixture of 2 gm. of metaphosphoric acid and 2 ml. of H_2SO_4 per liter. The ground sample was filtered through Whatman no. 2 paper and aliquots titrated for ascorbic acid by the usual method. Additional aliquots were treated as follows: a mixture containing 220 ml. of neutral potassium phosphate buffer (M/15), 20 ml. of 10 per cent. glucose and 20 ml. of bacterial suspension[1] was incubated in a water bath at 40° C. for approximately 15 minutes. To a 25-ml. aliquot of this mixture in a test tube were added 2 ml. of the turnip leaf extracts. The contents of the tube were rapidly mixed and two 5-ml. portions removed immediately for ascorbic acid titration by the usual method. At the end of a 15-minute incubation at 40° C., two additional 5-ml. samples were taken and titrated. The increase in reducing power of the cultures after 15 minutes incubation was ascribed to the conversion of dehydroascorbic acid to ascorbic acid by the bacteria.

CAROTENE

A detailed study of methods of carotene analysis of turnip greens was made, the results of which are to be presented in a separate paper (3). The essentials of the method finally selected are as follows: The leaves were killed by immersion in boiling water for 1/2 minute, and the pigments were extracted from the leaves by the method of MOORE and ELY (12). After filtration, water was added in the petroleum ether and ethyl alcohol extraction mixture, causing a separation of an alcoholic phase and a petroleum ether phase. The latter layer was removed, and the alcoholic phase was washed once with petroleum ether; two petroleum ether extracts were combined and washed twice with about 200 ml. of tap water. The petroleum ether extract, containing all the carotene and most of the chlorophylls and xanthophylls, was then dried over anhydrous sodium sulfate and made up to a given volume. Aliquots were then run through a starch-super-cell[2] column (2 parts of starch; 1 part super-cell by weight) which retained the chlorophylls and xanthophylls. The petroleum ether containing the carotenoid pigments was collected after passing through the column, made

[1] Prepared according to GUNSALUS and HAND (6). PROF. GUNSALUS kindly furnished the stock culture of *Bacterium coli*.

[2] Soluble starch, Indicator grade, from Eimer and Amend.

to an arbitrary volume, and an estimate of the carotene content obtained by use of a colorimeter standardized with β carotene. The method thus determines a group of carotenoid pigments consisting mostly of beta carotene.

Preliminary analyses and sampling techniques for ascorbic acid determinations

Before the turnips from the various experiments were analyzed, some preliminary work on methods of sampling was completed. Consideration was given to the possibility of harvesting the entire top, extracting for either carotene or ascorbic acid, and calculating the vitamin content on the fresh weight basis. This was undesirable since the extraction of the entire top of each individual plant would have involved the use of large quantities of reagents and because harvesting the entire top of plants in certain of the treatments would have involved the inclusion of some dead or partially dead leaves. Therefore, an investigation was made to determine what variation in vitamin content occurred between the leaves of various ages on a given plant and between the different parts of a given leaf in order to determine whether or not one leaf or leaf part might prove representative of the plant as a whole.

In all of this preliminary work, leaves of the Shogoin variety were used. Some of the plants were grown in soil in the greenhouse during the spring for the specific purpose of supplying this material. In part of the preliminary work, leaves were from plants grown in sand culture on a balanced nutrient solution (see treatment 13, figure 1) used in Experiment I.

On May 15, seventeen young leaves (approximately one to two inches in length) were selected from seventeen plants, six weeks old, grown in soil in the greenhouse. Each leaf was divided into basal, medial, and distal fractions of nearly equal area. The corresponding portions of the leaves of all plants were combined to give three samples which were analyzed for ascorbic acid. Similarly, eight older leaves were selected from the middle portion of some of the plants, and these were divided into three samples and analyzed for ascorbic acid as described above. Three of the oldest leaves

TABLE I

The effect of leaf age on ascorbic acid concentration (mg./100 gm. fresh weight) and distribution within the leaf*

Portion of leaf	Youngest leaves (17 leaves used)	Medium-aged leaves (8 leaves used)	Oldest leaves (3 leaves used)
	mg.	*mg.*	*mg.*
Basal end	144.5	107.7	88.9
Middle portion	149.2	115.1	109.1
Distal end	159.5	130.4	113.2
Average entire leaf	151.1	117.7	103.7

* Plants grown six weeks in soil in greenhouse in early spring.

which were of a uniform green color and showed no evidences of necrosis were also selected and treated as above. The results of the analyses are given in table I. There was, in general, an increase in ascorbic acid content from the basal to the distal end of the leaves, and from the oldest to the youngest leaf.

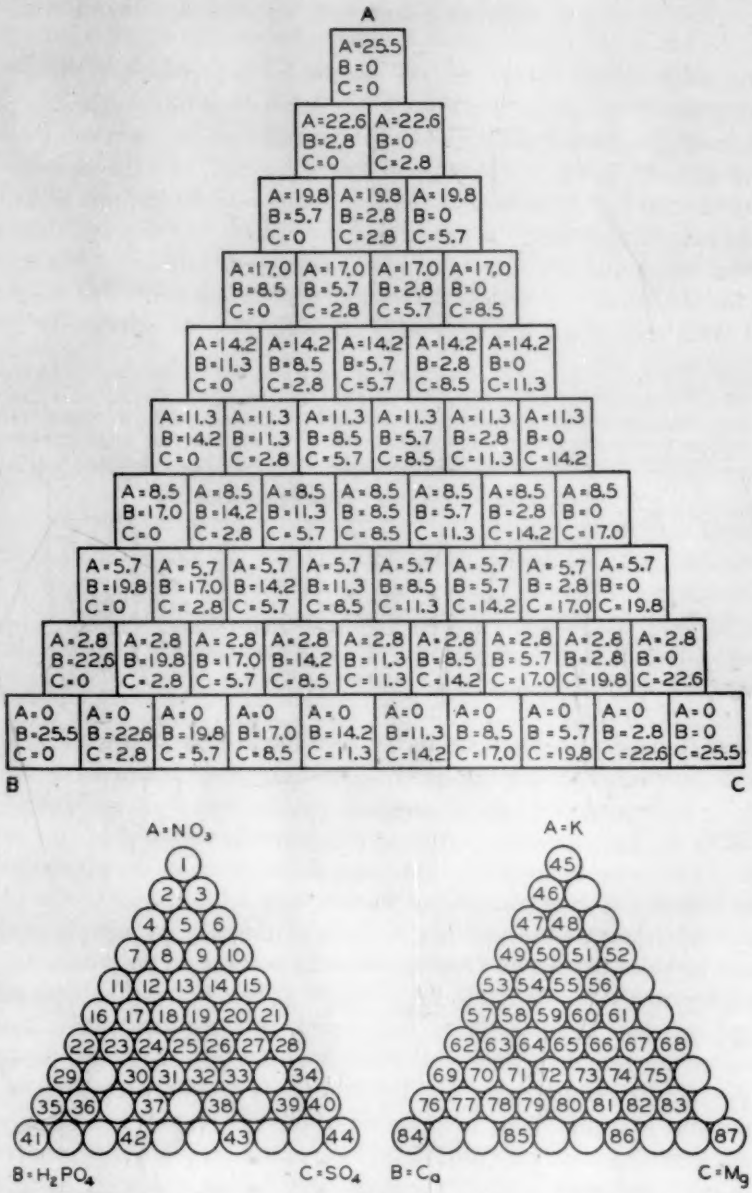

FIG. 1. Upper triangle represents relative proportions of macro-nutrient elements in milliequivalents per liter for 55 possible treatments. Left, Below: 44 combinations of varying anion concentrations. Right, below: 43 combinations of varying cation concentrations. Combinations indicated by treatment numbers.

Additional leaves from the middle portion of other plants in the greenhouse were harvested and each leaf divided longitudinally into two halves by the removal of the midrib which was discarded. Each half of each leaf was separately analyzed for ascorbic acid. It was found that, while there was considerable variation in ascorbic acid content between different leaves, the two halves of any individual leaf were in close agreement with each other.

One week before harvesting the plants in Experiment I for vitamin analyses, additional preliminary work was carried out on comparable plants grown in sand culture and supplied with the balanced nutrient solution used in treatment 13, figure 1. Five plants were selected, and the ascorbic acid content of each leaf from each of the five plants was determined separately. For the sake of simplicity in presenting the results, the average values for the three oldest and the three youngest leaves were calculated; an average value for the remaining middle leaves was also calculated. The results obtained with each plant are presented in table II. As previously found,

TABLE II

THE EFFECT OF LEAF AGE ON ASCORBIC ACID CONCENTRATION (MG./100 GM. FRESH WEIGHT). FIVE PLANTS HARVESTED FROM SAND CULTURE BALANCED NUTRIENT OF FIRST EXPERIMENT

POSITION OF LEAVES	NUMBER OF LEAVES	PLANT NUMBER					AVERAGE
		1	2	3	4	5	
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	
Oldest	3	191.4	166.0	182.4	222.9	201.5	192.8
Middle	2–9	223.2	199.0	210.7	199.7	190.0	204.3
Youngest	3	265.9	220.6	236.3	218.1	198.4	227.9
Average entire plant	.....	219.6	188.8	204.7	209.6	198.9	204.3

there was in general an increase in the ascorbic acid from the oldest to the youngest leaves. The average ascorbic acid content obtained for the five plants by averaging the values obtained for the leaves of various ages is almost exactly the same as the averages of the values obtained for the middle leaves. It thus seems apparent that the middle leaves on the plants may be used as a basis for determination of the ascorbic acid content of the plants.

To check further as to whether or not a representative sample could be obtained by selecting leaves from the middle portion of the plant, another preliminary analysis was made from plants grown in sand culture on the balanced nutrient solution from Experiment I (treatment 13). Instead of selecting the youngest leaves and the oldest leaves on the plant, the leaves from the middle portion were divided into three groups, according to age. The youngest group of these were leaves which were more than four inches in length and thus nearly expanded, and the oldest leaves of this group were all of a uniform green color. The remaining leaves, those of the middle-aged group, represented leaves of an age comparable to those used in all the subsequent analyses reported in the several experiments. The results of the analyses of these leaves were as follows:

Average ascorbic acid content of 20 young leaves 202.0 ± 7.94 mg./100 gm.
Average ascorbic acid content of 40 medium-aged leaves 186.7 ± 4.59 mg./100 gm.
Average ascorbic acid content of 20 older leaves 194.0 ± 6.03 mg./100 gm.

No significant differences in ascorbic acid content were found among the samples of these medium-sized, medium-aged leaves of the plant. The agreement between the three groups was within 8 per cent.

At the time these analyses were made, a number of leaves of the middle-aged group were collected at 9 A.M. and each leaf divided into halves longitudinally by the removal of the midrib. The indivdual half leaves were placed in Petri dishes between layers of moist filter paper and set in the laboratory. One-half of each leaf was analyzed at once and the analyses completed by 11 A.M., and the remainder were analyzed at 10 P.M. on the same day. The latter set had been divided into two groups; one stored at room temperature, and the other in the ice box at 40° F. Both of these groups gave the same ascorbic acid values regardless of storage temperatures, and therefore the results are presented together. The results of these analyses were as follows:

Average ascorbic acid content of 40 leaves at 11 A.M. 186.7 ± 4.59 mg./100 gm.
Average ascorbic acid content of 40 leaves at 10 P.M. 190.5 ± 3.12 mg./100 gm.

Essentially the same ascorbic acid content was obtained with samples analyzed immediately after harvesting or later in the day.

In another preliminary experiment, leaves of middle age were harvested at different times during the day from plants grown outside the greenhouse in sand culture (treatment 13 of Experiment I). The results are presented in table III, both on fresh and dry weight basis. The samples collected at 9 A.M. and 12:30 P.M. agree with each other, while those collected at 4:30 P.M. were slightly higher on a fresh weight basis but not on a dry weight basis.

As a result of this preliminary work, the following procedure was used in sampling the plants in the experiments to be described later. At 9 A.M. medium-aged leaves from individual plants of a given experiment were harvested and placed between layers of moist filter paper contained in Petri dishes. As soon as possible thereafter, each leaf was weighed, the midrib

TABLE III

ASCORBIC ACID CONTENT OF LEAVES HARVESTED AT DIFFERENT TIMES DURING THE DAY. PLANTS GROWN IN SAND CULTURE WITH BALANCED NUTRIENT SOLUTION OF EXPERIMENT I

TIME HARVESTED	NUMBER OF LEAVES	PERCENTAGE DRY WT.	AVERAGE ASCORBIC ACID	
			PER 100 GM. FRESH WT.	PER GM. DRY WT.
		%	*mg.*	*mg.*
9:00 A.M.	10	15.9	205 ± 5.7	129
12:30 P.M.	10	15.3	209 ± 1.7	137
4:30 P.M.	10	16.8	223 ± 5.9	133

excised, and the blade divided longitudinally into halves. Each half was then weighed and again placed in a Petri dish between moist layers of paper. As soon thereafter as possible, one half was analyzed for ascorbic acid, and the other half partially dried in a forced-draft oven at 65° C. and later completely dried in a vacuum oven at 60° C. The percentage of moisture of each leaf was then calculated from fresh and dry weights of the left half of the leaf, and then these dried samples were set aside for subsequent thiamine and nicotinic acid determinations. In most analyses, the ascorbic acid determinations were made within a few hours after sampling; in some, however, as much as six or eight hours elapsed. All analyses were completed on the same day that the leaves were harvested.

Preliminary work on carotene analyses

Preliminary work on carotene analyses was carried out in a manner similar to that described above for ascorbic acid. The results of this work are presented in tables IV to VI. The carotene was distributed fairly uniformly throughout the leaf, and the leaves of different ages had nearly the same carotene concentration (table IV), although the oldest leaves were somewhat lower in carotene than were the other age groups. There was some indication that the carotene content, both on the fresh weight and dry weight basis, decreased during the middle of the day (table VI).

TABLE IV

The effect of leaf age on carotene concentration (μ carotene per gm. fresh weight) and distribution within the leaf*

Portion of leaf	Youngest leaves (15 leaves used)	Medium-aged leaves (8 leaves used)	Oldest leaves (5 leaves used)
	μ	μ	μ
Basal end	101.1	107.2	93.9
Middle portion	95.9	100.5	87.7
Distal end	103.2	115.3	85.1
Average entire leaf	99.7	107.3	88.9

* Plants grown six weeks in soil in greenhouse in early spring.

TABLE V

Effect of leaf age on carotene concentration (μ carotene per gm. fresh wt.). Five plants harvested from sand cultures, complete nutrient of first experiment

Position of leaves	Number of leaves	Plant number					Average
		1	2	3	4	5	
		μ	μ	μ	μ	μ	
Oldest	3	34.3	38.5	29.7	41.5	28.3	34.5
Middle	3–5	39.9	34.5	33.0	40.4	38.6	37.3
Youngest	3	40.6	41.0	35.7	38.9	48.4	40.9
Average entire plant	.....	37.6	36.9	34.2	.....	38.1	36.7

TABLE VI

CAROTENE CONTENT OF LEAVES HARVESTED AT DIFFERENT TIMES DURING THE DAY. PLANTS GROWN IN SAND CULTURE, COMPLETE NUTRIENT, EXPERIMENT I

TIME HARVESTED	NUMBER OF LEAVES	PERCENTAGE DRY WT.	AVERAGE CAROTENE, μ/GM.	
			FRESH WT.	DRY WT.
		%	μ	μ
9:00 A.M.	9	14.6	46.5 ± 1.8	318.5
12:30 P.M.	10	16.9	40.2 ± 2.1	237.9
4:30 P.M.	10	17.1	55.8 ± 3.4	326.3

Following the same procedure that was used in the preliminary ascorbic acid work, 72 leaves from the middle portions of the plants were selected and divided into two groups: one consisting of 36 of the younger leaves and the other of 36 of the older leaves. The carotene analyses were as follows:

Average carotene content of 36 younger leaves, 49.5 ± 1.04 mg./gm.
Average carotene content of 36 older-aged leaves, 51.0 ± 0.82 mg./gm.

Thus, within the medium-aged group there was very little difference between the younger and the older leaves.

When leaves were harvested in the morning and one-half analyzed at once, they had essentially the same carotene content as comparable half leaves analyzed later in the afternoon:

Average carotene content of 36 leaves analyzed in A.M., 50.1 ± 0.92 mg./gm.
Average carotene content of 36 leaves analyzed in P.M., 50.4 ± 0.96 mg./gm.

As a result of this work, the same procedure was followed in harvesting samples for carotene analysis as has been described for the ascorbic acid determinations.

Experiment I

By measuring the variations in vitamin content which accompany variations in fertility, an estimate of the relative importance of fertilization on nutritive value may be obtained. Because of the amount of work involved it was arbitrarily decided to limit the work to a study of variations in fertility associated with differences in the supply of the macronutrients (calcium, magnesium, phosphorus, nitrogen, potassium, and sulfur). The variations in fertility of the various treatments was judged by the appearance and yield of the plants produced.

Plants were grown in both sand and soil culture. Through the use of sand culture, it was possible to supply known amounts of mineral nutrients in the various treatments over a wide range. By the selection of soils of low and moderate fertility and through fertilization treatments somewhat analogous to certain of the treatments in the sand cultures, some estimate could be made of the degree to which the results in sand culture, where the supply of mineral nutrients was accurately known, could be interpreted in terms of field practice.

Two soils relatively low in fertility were obtained near Ithaca. Samples of both were brought to the laboratory and placed in crocks comparable to those used in the sand culture work and fertilized in the various ways described below. Field experiments were also conducted at each of the locations where the soils were obtained. At each of these locations pots of each soil were set up and fertilized with one of the balanced fertilizers (treatment 20, Table VII) used in the soil work.

Procedure

The design and procedure used in the sand culture work with turnips was essentially the same as that used in work with tomatoes (**7**). The design was that of a randomized block (**4**) with eighty-seven treatments and four replications. Each replication consisted of a two-pot row, and each pot contained two plants. The replications were randomized by the use of Tippett's randomization tables (**17**). Thus, each treatment consisted of 8 pots with 16 plants. The west plant from each pot was used for the vitamin analyses and the other for growth and dry weight data.

The method of preparing the nutrient solutions has been previously described (**7**). Eighty-seven nutrient solutions were used, and all contained the following concentrations of microelements: 0.5 p.p.m. of B as HBO_3, 0.5 p.p.m. of Mn as $MnCl_2$, 0.05 p.p.m. of Zn as $ZnSO_4$, 0.02 p.p.m. of Cu as $CuSO_4$, and 5.0 p.p.m. of Fe as ferric citrate. In forty-four of the solutions the proportions of nitrate, phosphate, and sulfate were varied, while each contained 12.0 m.e./l. of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium. The proportions of potassium, calcium, and magnesium were varied in the remaining forty-three of these solutions, each of which contained 12.0 m.e./l. of nitrate, 4.5 m.e./l. of phosphate, and 9.0 m.e./l. of sulfate (fig. 1). For convenience, the nutrient solutions are represented in cation and anion triangles, and treatment numbers are assigned to those solutions which were used. For purposes of discussion the treatments devoid of one or more ions will be designated as deficient.

For the soil experiments several tons of soil were obtained from the top six inches of each of two fields near the laboratory. One of these soils was a Dunkirk silty clay loam located at Caldwell field; the other was a Mardin silt loam located at Mt. Pleasant Field. Both of these fields were under the supervision of the Cornell University Agronomy Department and, as far as it is known, no differential fertilizer treatments had been applied for the past twenty years. On comparable adjacent fields, plants were known to respond markedly to applications of lime and fertilizers. The soil from both fields was placed in two-gallon crocks, 15 pounds per crock. Five pounds of sand were placed in the bottom of each crock before adding the soil and a glass tube extended from the layer of sand to the surface of the soil in all treatments except one (the puddled soil). Before adding the soil to the crock, the fertilizer and lime (if applied) were mixed thoroughly with the soil of each pot. In those treatments calling for application of manure, alfalfa

TABLE VII

FERTILIZER TREATMENTS APPLIED TO SOIL-POTS AND FIELD PLOTS OF EXPERIMENT I

TREATMENT	LIME PER ACRE	INORGANIC FERTILIZER PER ACRE						APPLICATION TO†				REMARKS
								POT-CULTURES		FIELD PLOTS		
		Ca	K	Mg.	N	P_2O_5	S	DUNKIRK	MARDIN	DUNKIRK	MARDIN	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
1	.....	.....	.....	.....	.....	.....	.....	+	+	.....	.....	
2	2	.....	.....	.....	.....	.....	.....	+	+	+	+	
3	2	.....	.....	.....	.....	.....	.....	+	.....	.....	.....	Soil puddled
4	10	.....	.....	.....	.....	.....	.....	+	+	+	+	
5	10	.....	.....	.....	.....	.....	.....	.....	.....	+	+	Added microelements*
6	2	.....	.....	.....	.....	.....	.....	.....	.....	+	+	Added microelements*
7	2	29.6	43.6	13.5	20.5	.....	29.7	+	+	+	+	
8	2	29.6	43.6	13.5	.....	.....	59.3	+	+	+	+	
9	2	29.6	43.6	13.5	41.0	.....	.....	+	+	+	+	
10	2	29.6	43.6	13.5	.....	105.0	29.7	+	+	+	+	
11	2	29.6	43.6	13.5	.....	210.0	.....	+	+	+	+	
12	2	29.6	43.6	13.5	20.5	105.0	.....	+	+	+	+	
13	2	37.0	.....	22.5	16.4	63.6	17.8	+	+	+	+	
14	2	.....	.....	45.0	16.4	63.6	17.8	+	+	+	+	
15	2	74.0	.....	.....	16.4	63.6	17.8	+	+	+	+	
16	2	37.0	72.0	.....	16.4	63.6	17.8	+	+	+	+	
17	2	.....	144.0	.....	16.4	63.6	17.8	+	+	+	+	
18	2	.....	72.0	22.5	16.4	63.6	17.8	+	+	+	+	
19	.....	24.7	48.0	15.0	16.4	63.6	17.8	+	+	.....	.....	
20	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
21	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Added microelements*
22	10	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
23	10	24.7	48.0	15.0	16.4	63.6	17.8	.....	.....	+	+	Added microelements*
24	2	29.6	43.6	13.5	13.6	70.0	19.8	+	+	+	+	
25	2	.....	.....	.....	.....	.....	.....	+	+	.....	.....	Ash of 10 tons of manure
26	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	.....	.....	Ash of 10 tons of manure per acre
27	2	.....	.....	.....	.....	.....	.....	+	+	+	+	Ten tons of manure per acre
28	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Ten tons of manure per acre

* Twenty-five pounds per acre each of manganese sulfate, copper sulfate, zinc sulfate, and boric acid.
† The symbol + indicates that the fertilizers to the left have been added.

meal, or ash of organic material the application was made by mixing the material with the soil of the middle third of the pot to simulate the position of a plow-down incorporation.

Each treatment with each soil consisted of ten pots with two pots in each of five blocks. In each block all pots were randomized by use of TIPPETT'S randomization tables. Thus, there was a total of 490 pots, with 24 treatments on each of two soils and one additional treatment on one soil. Most treatments were identical for the field plots and the soil pots, but a few of them were applied to the soil pots and not to the field plots and vice versa. Thus, a total of 28 treatments was used and each, regardless of where it was applied, was given a number.

The field areas were 100×62 feet, and these were laid out in 31 rows, extending the length of the field and two feet apart. Alternate rows of plants, with the exception of the outermost two rows, were used as experimental rows. A given treatment was applied to nine feet of a given row, and on any particular row the treatments were separated from one another by a one-foot portion which was not fertilized or harvested. The field was divided into six equal blocks in such a manner that each row extended through two blocks. Each of 25 treatments occurred once in each of the six blocks. The treatments were randomized within a given block by the use of TIPPETT'S randomization tables (**17**). The fertilizers were applied just before planting and worked into the top two inches of the soil for six inches on either side of the row.

Seeds were planted in the field plots on May 1. On June 9 the seedlings were well established, and the plants were thinned so that there remained one plant in every four inches of each row. Seeds from the same lot were planted in the greenhouse on May 1 in sand contained in pots 4×7 inches. The sand was thoroughly moistened with nutrient solution, and, as the seedlings germinated, they were watered about twice a week with complete nutrient solution (see treatment 13, fig. 1). A sufficient number of seeds was planted in each pot to assure germination of five seeds per pot. As the seedlings became established, plants were removed from each pot so as to leave two uniform seedlings in each. On May 27, when the seedlings were 26 days old, they had approximately 4 foliage leaves and the largest leaf on each plant was approximately 3–4 inches long. At this time, the seedlings were transplanted to two-gallon crocks. Seven hundred and twenty of these crocks contained white quartz sand and 490 contained soil to which the various fertilizer mixtures had been applied. Two seedlings were transplanted to each crock and the plants watered with distilled water. The plants in sand were watered the next day with the respective nutrient solutions as previously described; this was repeated twice each week during the early stages of growth and three times each week during later stages when the plants were fairly large. A sufficient amount of nutrient solution was applied at each watering so that an appreciable amount of liquid dripped from the bottom of the pot. The plants in soil pots were watered with dis-

tilled water as needed, which was only occasionally since rain during the season supplied most of the requirements. Subsequent to transplanting, the plants were grown in the greenhouse for a period of twelve days; on June 8 all pots were removed to a plot of ground outside and arranged in the same design as was used in the greenhouse.

On June 25, at which time the first samples for vitamin analysis were taken, the plants in the more favorable treatments were fairly large and had many leaves. The plants in these treatments had reached a stage at which they might be harvested for greens. On June 25, 26, and 27, samples were collected for carotene analysis from the sand cultures, the soil pots, and the fields, respectively; on June 30 ascorbic acid samples were taken from the sand cultures and soil-pots. On July 2, plants in both fields were sampled for ascorbic acid. On July 5, another set of samples for ascorbic acid analysis was taken from the sand cultures. For any given vitamin determination, one leaf was taken from one of the plants in each pot; two leaves from the two pots of a particular treatment in any given block were combined as a single sample. The two leaves were divided longitudinally into halves by the removal of the midrib and weighed. One half was analyzed for vitamin content and the other half used for dry weight determinations. The remaining plants which had not been used for vitamin analyses were harvested according to the following schedule: soil-pots on July 6; sand cultures on July 7; Caldwell field plots on July 8; and Mt. Pleasant field plots on July 9. At the harvest, the plants were pulled up. The tops were separated from the roots and the roots washed thoroughly, removing all fine, fibrous roots and retaining the main tap root. Fresh weight determinations were made on both roots and tops. The tops were then washed by dipping in three successive containers of distilled water to remove dust. The length and diameter of the enlarged portion of the tap root were measured. The tops and roots were then dried for 48 hours in a forced draft oven at approximately 80° C. and weighed.

Results

During germination and early seedling development, growth was more rapid in the greenhouse than in the field. During the middle and latter part of June, however, the plants in the field, in general, grew much more rapidly and at harvest time were considerably larger than the plants in sand culture or pots. The field plants, unlike those in the greenhouse, were never transplanted. The seedlings in the fields were exposed to the natural fluctuations in temperature, while those at the laboratory were subjected to greenhouse conditions. During early May, the field plants were exposed to below freezing temperatures on several nights. At the time of harvest, the stems of many plants at both fields had elongated and some were in flower. In selecting plants for vitamin analysis at the fields, two plants of each treatment of each block were chosen which showed no signs of bolting. The plants in the pots containing soil at each field resembled closely the plants at the laboratory. These were placed in the field at the time the plants at the laboratory were removed from the greenhouse.

Detailed visual observations were made on the plants at successive stages in their development. In addition, counts were made of the number of leaves and measurements taken of the total leaf length of all plants in certain selected treatments. These data were collected on June 11 and 12 and again one week later. At the time of the final harvest, in addition to determination of fresh and dry weights of roots and tops, measurements were made of the length and diameter of the enlarged portion of the tap root of each individual root. Calculations of the percentage of moisture and root-top ratios were also made. These data are too voluminous to be included here. They all serve to establish the fact that the various treatments employed produced great variations in the growth and development of the plants. Only the data on the fresh weights of the tops of the plants are presented. A brief description of the appearance of the plants is included with the discussion of the results. The growth data for the sand cultures are given in figure 2 and for the soil treatments in table VIII. These growth data inadequately demonstrate the differences between treatments, since the type of growth, amount of chlorosis, number of dead leaves, and general appearance were correlated with differences in treatment.

The average value given for the fresh weight of the tops receiving a given treatment is an average of eight plants. The analysis of variance of the growth data for the plants in sand cultures shows that significant differences between treatments existed within both triangles (at the 1 per cent. level). In the anion triangle (fig. 2) very little growth was obtained in those treatments deficient in nitrate and phosphate, and those treatments deficient in sulfate produced much less growth than the more nearly optimum treatments. In the nitrate-deficient treatments the plants were chlorotic and reddish in color. The plants of the sulfate-deficient treatments were chlorotic, especially between the veins, with a red coloration on the under side of the leaves; those of the phosphate-deficient treatments were of a darker green color than plants in the more nearly optimum treatments with some bronze coloration in the older leaves. Increasing amounts of growth were obtained with increasing supplies of nitrate, up to and including those treatments which received 11.3 milliequivalents of nitrate per liter of nutrient. There was an increase in green coloration correlated with this increasing growth. In the cation triangle (fig. 2) little growth was obtained after transplanting in the treatments deficient in potassium and calcium; growth was significantly less than the optimum in treatments deficient in magnesium. All plants in the treatments deficient in magnesium and potassium were distinctly chlorotic. The chlorosis was particularly apparent between the veins in the magnesium-deficient treatments, while it was more uniformly distributed over the entire leaf in the potassium-deficient series. While the plants in the calcium-deficient series produced but little growth, they were not chlorotic. Treatments 67, 75, and 83, which contained little potassium or calcium and relatively large amounts of magnesium (fig. 1), produced little growth and were somewhat chlorotic. Significant differences between

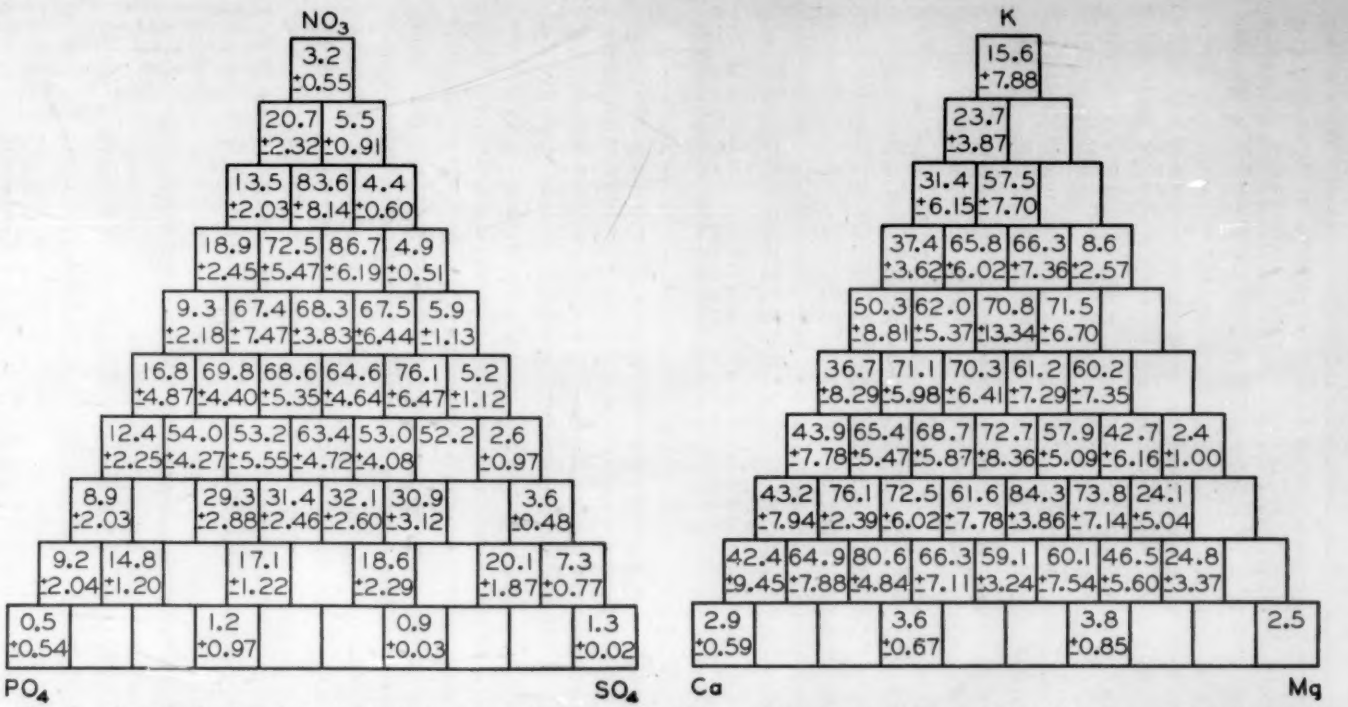

FIG. 2. Average fresh weight of turnip tops in sand cultures of first experiment giving treatment means (gm. per plant) together with the standard error. Each mean represents the average of eight plants.

Anion triangle (left). The "F" values from the variance analysis are: treatments 55.14† (43 degrees freedom); within blocks 2.20 (1 d.f.); and between blocks 3.69* (3 d.f.). No first order interactions were significant.

Cation triangle (right). The "F" values from the variance analysis are: treatments 10.58† (42 degrees of freedom); within blocks 1.56 (1 d.f.); and between blocks 5.76† (3 d.f.). No first order interactions were significant.

* Significant at the 5% level.

† Significant at the 1% level.

TABLE VIII

FRESH WEIGHTS, CAROTENE CONTENT, AND ASCORBIC ACID CONTENT OF TURNIPS GROWN IN SOIL OF EXPERIMENT I

TREATMENT	FRESH WT. OF TOPS (GM./PLANT) POT-CULTURES		FRESH WT. OF TOPS (KG./9′ ROW) FIELD PLOTS		CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) POT-CULTURES[1]		CAROTENE (μ/100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[2]		ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) POT-CULTURES[3]		ASCORBIC ACID (MG./100 GM. FRESH WT. OF LEAVES) FIELD PLOTS[4]	
	DUNKIRK	MARDIN	DUNKIRK	MARDIN	DUNKIRK	MARDIN	DUNKIRK	MARDIN	DUNKIRK	MARDIN	DUNKIRK	MARDIN
	gm.	*gm.*	*kg.*	*kg.*	μ	μ	μ	μ	*mg.*	*mg.*	*mg.*	*mg.*
1	19.9	6.1	.....	.....	60.5 ± 2.05	56.2 ± 9.10	....	....	288 ± 12.0	281 ± 13.1	....	....
2	23.8	18.1	0.57	0.47	58.8 ± 2.61	60.4 ± 5.63	64.7 ± 3.15	75.9 ± 4.18	273 ± 10.8	264 ± 7.7	195 ± 8.4	187 ± 17.1
3	19.6	.....	.....	.....	57.2 ± 3.36	....	....	....	292 ± 24.5	....	....	....
4	34.2	22.1	0.50	0.73	62.6 ± 8.59	51.8 ± 4.95	68.8 ± 3.08	75.9 ± 2.51	252 ± 9.0	259 ± 7.8	183 ± 4.9	182 ± 11.8
5	.....	.....	0.39	0.71	....	....	66.6 ± 2.49	72.8 ± 4.18	....	....	194 ± 14.2	170 ± 6.6
6	.....	.....	0.71	0.73	....	....	67.6 ± 3.29	74.9 ± 4.23	....	....	171 ± 4.5	173 ± 6.2
7	25.0	24.3	0.65	0.80	53.1 ± 5.69	69.8 ± 3.10	72.5 ± 1.93	74.5 ± 2.85	263 ± 22.7	287 ± 16.1	186 ± 7.4	171 ± 6.9
8	25.9	17.3	0.53	0.71	62.0 ± 5.04	58.8 ± 2.90	76.2 ± 2.00	76.3 ± 2.96	275 ± 19.2	260 ± 7.9	187 ± 7.3	186 ± 13.8
9	29.0	21.4	0.63	0.63	54.7 ± 4.17	58.0 ± 4.08	74.9 ± 3.84	82.3 ± 3.88	266 ± 13.0	296 ± 15.0	182 ± 9.9	192 ± 7.3
10	29.1	19.9	2.38	1.01	61.3 ± 5.02	63.8 ± 1.70	64.7 ± 4.27	77.1 ± 4.52	303 ± 19.6	270 ± 12.5	179 ± 7.6	197 ± 4.9
11	35.1	21.3	3.05	1.08	63.3 ± 3.42	46.5 ± 4.25	72.0 ± 3.65	73.9 ± 4.30	283 ± 13.2	265 ± 6.8	171 ± 12.6	170 ± 21.6
12	36.1	23.5	2.49	1.52	64.0 ± 2.63	64.6 ± 2.51	72.5 ± 2.26	78.3 ± 1.85	262 ± 12.2	267 ± 13.8	173 ± 9.6	191 ± 10.0
13	30.4	21.9	1.42	1.03	65.7 ± 2.76	66.9 ± 3.35	77.1 ± 6.65	82.0 ± 3.66	276 ± 20.1	306 ± 4.8	198 ± 10.5	185 ± 15.3
14	24.9	22.3	1.21	0.92	59.7 ± 12.08	68.3 ± 4.15	73.9 ± 2.55	74.7 ± 2.85	270 ± 6.9	265 ± 19.4	167 ± 10.6	179 ± 10.3
15	29.9	21.1	1.70	0.81	51.9 ± 6.15	61.1 ± 1.71	75.5 ± 4.32	77.5 ± 3.22	274 ± 17.7	269 ± 11.4	193 ± 11.6	172 ± 8.1
16	31.7	23.2	2.25	1.62	62.7 ± 5.79	52.7 ± 8.46	79.3 ± 2.68	75.6 ± 2.08	275 ± 14.9	282 ± 12.8	185 ± 19.0	187 ± 7.6
17	33.7	24.1	2.02	1.65	59.7 ± 3.94	53.8 ± 3.35	66.4 ± 1.10	81.1 ± 5.55	313 ± 9.4	277 ± 8.2	188 ± 11.4	191 ± 8.7
18	29.8	24.5	2.60	1.58	61.6 ± 5.19	63.6 ± 2.22	72.2 ± 3.78	82.2 ± 2.57	299 ± 15.1	276 ± 12.5	194 ± 7.8	190 ± 8.2
19	23.2	11.3	.....	.....	65.0 ± 4.58	62.8 ± 2.92	....	....	267 ± 7.9	267 ± 18.6	....	....
20	32.2	20.9	2.19	1.49	58.2 ± 11.88	60.5 ± 4.15	74.3 ± 1.58	76.6 ± 2.51	303 ± 3.5	271 ± 10.1	174 ± 21.8	189 ± 13.6
21	33.6	22.2	1.45	1.06	69.6 ± 1.42	56.2 ± 4.75	71.6 ± 1.10	74.3 ± 4.19	281 ± 6.2	269 ± 13.3	183 ± 4.3	190 ± 8.9
22	33.4	31.6	2.45	1.53	64.2 ± 2.22	53.3 ± 7.64	76.2 ± 2.28	82.9 ± 1.68	280 ± 12.5	247 ± 26.8	191 ± 7.2	192 ± 12.3
23	.....	.....	1.56	1.51	....	....	70.4 ± 2.67	79.1 ± 1.90	....	....	186 ± 10.3	179 ± 5.7
24	30.0	17.8	2.28	1.39	67.7 ± 3.95	67.3 ± 4.34	72.1 ± 3.84	72.4 ± 1.58	270 ± 13.5	277 ± 15.7	163 ± 14.3	185 ± 17.1
25	34.6	24.3	.....	.....	64.7 ± 2.26	51.2 ± 2.35	....	....	293 ± 7.6	246 ± 16.6	....	....
26	34.9	29.3	.....	.....	63.8 ± 4.19	51.6 ± 4.11	....	....	265 ± 12.3	288 ± 12.2	....	....
27	40.9	21.4	2.91	1.39	57.8 ± 5.27	51.9 ± 3.12	67.4 ± 4.18	70.5 ± 4.15	273 ± 12.5	259 ± 4.6	169 ± 16.6	162 ± 10.8
28	33.8	29.9	3.25	1.67	60.3 ± 3.69	54.0 ± 3.61	70.0 ± 2.97	71.0 ± 3.88	258 ± 10.1	240 ± 9.5	163 ± 10.4	172 ± 6.9
	Average vitamin values				61.2	58.5	71.6	76.6	278.2	270.3	181.5	182.2
20	Dunkirk soil in pots at fields				....	....	82.7 ± 0.92	86.7 ± 4.86	.....	.....	193 ± 6.8	215 ± 8.5
20	Mardin soil in pots at fields				....	....	70.2 ± 2.26	79.0 ± 2.71	.....	.....	220 ± 4.1	250 ± 10.2

"F" values for variance analyses are: (see above superscripts 1 to 4 incl.)

1. Treatments = 1.29, between blocks = 14.28,† soils = 3.93,* treatment × soils = 1.86.* No other first order interactions were significant.
2. Treatments = 2.22,† between blocks = 0.25, field = 30.47,† field × blocks = 8.70.† No other first order interactions were significant.
3. Treatments = 1.33, between blocks 11.22† soils = 3.05. No first order interactions were significant.
4. Treatments = 1.39, between blocks = 0.09, field = 1.26. No first order interactions were significant.

* Significant at the 5% level. † Significant at the 1% level.

blocks in both triangles are indicated since the "F" value in one case exceeds requirements for odds of 99:1 and in the other case satisfies the requirements for odds of 19:1. This indicates that variations in environmental conditions within the small experimental field produced significant effects upon growth. The field where the pots were located was adjacent to two buildings but was not shaded; however, there may have been some light reflection from the sides of the buildings to the field, and wind movements may have been modified.

Relatively great differences in growth were obtained as a result of soil treatment (table VIII). Differences in color and development were not as striking as between the sand culture treatments, although those treatments containing no phosphorus or excess lime produced plants, both in pots and in the field, which were a light green color, and many exhibited a mottled appearance. The more nearly optimum soil treatment produced plants of a deeper green color than those of the sand cultures. In general, those soil treatments involving nitrogen, phosphorus, or manure produced the most growth.

The average ascorbic acid values for the sand cultures each represent a total of eight analyses (fig. 3). Four of these analyses were made on June 30, and each of the four represented a composite sample taken from leaves of two plants in a particular block. The other four analyses were made from the same plants a few days later. The analysis of variance for the anion triangle shows that significant differences in ascorbic acid (at the 1 per cent. level) could be attributed to treatments, replications, and to replication-treatment and replication-date-of-harvest interactions. In the cation triangle, significant differences in ascorbic acid could be attributed to treatment and to replication and to a replication-date-of-harvest interaction. This would indicate that the treatments in both triangles produced significant effects upon ascorbic acid content as did also environmental variables associated with differences in location in the small experimental field. Differences with respect to date of harvest were associated with replication since a significant interaction was noted in both triangles. In the anion triangle, the replication-treatment interaction indicates an effect of treatment which is greater at certain locations than at others.

Those treatments which were deficient in sulfate, nitrate, and potassium produced plants which were significantly lower in ascorbic acid than the others, and there were increasing amounts of ascorbic acid with increasing nitrate supplies in the anion triangle in those treatments receiving less than 8.5 milliequivalents of nitrates per liter. One of the treatments deficient in calcium only produced plants of low ascorbic acid values while the other did not. Deficiencies of phosphorus and magnesium had little effect upon ascorbic acid content.

The differences in ascorbic acid content between the different replications noted here are in agreement with previous results obtained with tomatoes in which differences in ascorbic acid content could be associated with differ-

3. Treatments = 1.35, between blocks 11.22† soils = 3.05. No first order interactions were significant.
4. Treatments = 1.39, between blocks = 0.09, field = 1.26. No first order interactions were significant.
* Significant at the 5% level. † Significant at the 1% level.

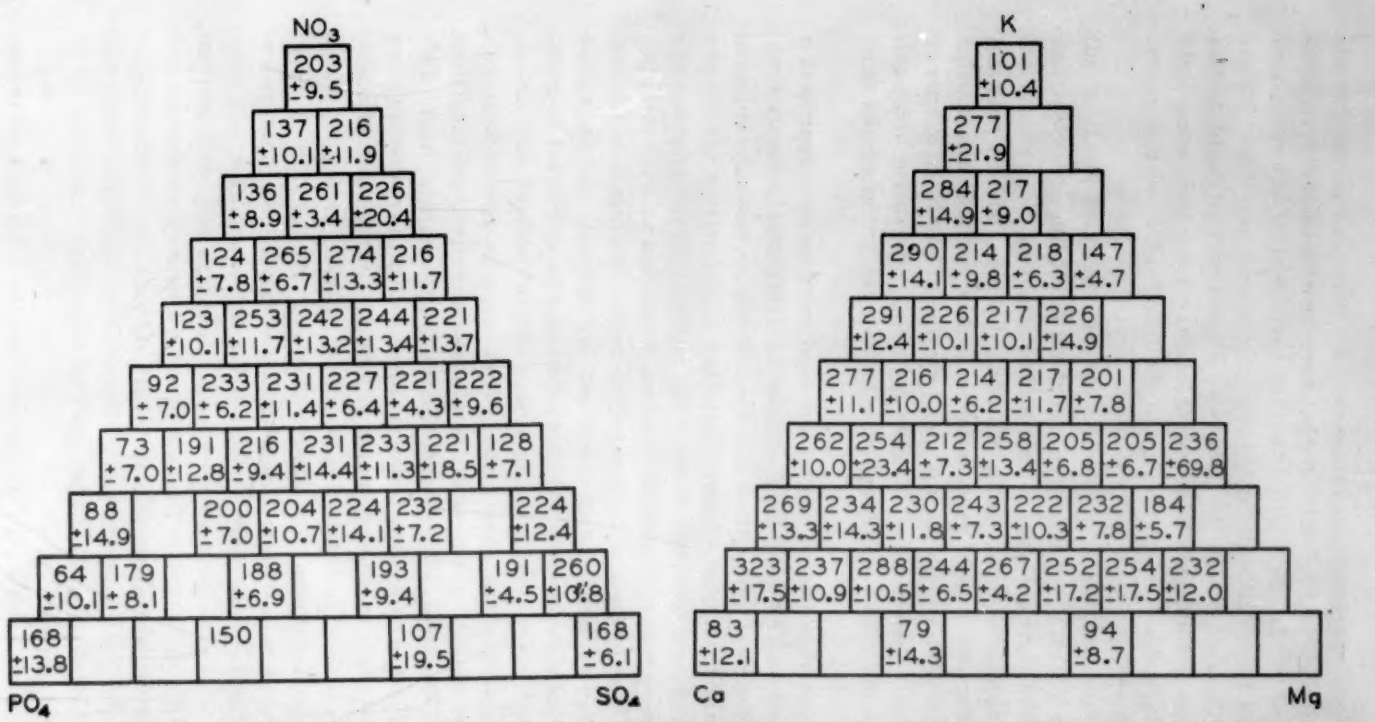

FIG. 3. Ascorbic acid values (mg. per 100 gm. fresh weight) of turnip greens of sand culture in first experiment giving treatment means with their standard errors.

Anion triangle (left). The "F" values from the variance analysis are: treatments 33.44* (43 degrees of freedom); between blocks 7.23* (1 d.f.); date of harvest 1.79 (1 d.f.); treatments × blocks 1.62* (129 d.f.); blocks × dates was not significant.

Cation triangle (right). The "F" values from the variance analysis are: treatments 19.93* (41 degrees of freedom); between blocks 9.98* (3 d.f.); date of harvest 1.17 (1 d.f.); blocks × date 4.85* (3 d.f.). No other first order interactions were significant.

* Significant at the 1% level.

ences in position in small fields. Significant differences in ascorbic acid content could be associated with replication interacting with the date of harvest indicating that significant variations in ascorbic acid content occurred within a short period of time in certain blocks.

From an analysis of variance of the data on ascorbic acid content of the plants in the pots containing soil, significant differences could be associated only between replications. There were no significant differences as a result of treatment, nor were there differences associated with the two types of soils used, nor were any interactions evidenced. In the field plots no significant differences were evident, indicating no effect of treatment or position in the field, nor were there differences between the two fields.

In general, the average carotene values (fig. 4, table VIII) given represent four analyses for any given treatment. Each analysis was obtained from a combined sample of two plants in any given block. The analysis of variance demonstrated significant differences in both the anion and cation triangles of the sand cultures associated with treatment, and no significant differences between replications were noted. The treatments deficient in sulfate, magnesium, potassium, and calcium were significantly lower in carotene than the other treatments, and increasing amounts of carotene were obtained with increasing supplies of nitrate in those treatments containing less than 11.3 milliequivalents of nitrate per liter. The treatments deficient in phosphate contained as much carotene as any of the other treatments of the anion triangle. Low values for carotene were obtained in treatments 75 and 83 (fig. 1) which also produced poor growth. All plants which were significantly lower in carotene were also chlorotic. While those plants growing on the phosphorus-deficient treatments produced relatively little growth, the leaves were of a very deep green color and rich in carotene. This would indicate that the carotene content is not necessarily directly correlated with the amount of growth produced since these phosphorus-deficient plants produced less than one-tenth the amount of growth produced in some of the more optimum treatments of the same carotene content, and less than one-third the growth of some of the magnesium-deficient series which had much less carotene.

In the soil-pot cultures, significant differences in carotene could be attributed to differences between the replications, but not to treatment. Differences which could be correlated with the soils used were significant at the 5 per cent. level, and the treatment-soil interaction was also significant at the 5 per cent. level indicating that possible effects of treatment were dependent upon the soil used. In the field plots significant differences in carotene could be attributed to treatment, and significant differences were also apparent between the two fields. A field-replication interaction is also highly significant, indicating that the effects due to replication are greater in one field than in the other. The plants at the Dunkirk field were lower in carotene than at the Mardin field. The values for carotene at the fields range from 64.7 to 79.3 and from 70.5 to 82.9 gamma per 100 grams, respec-

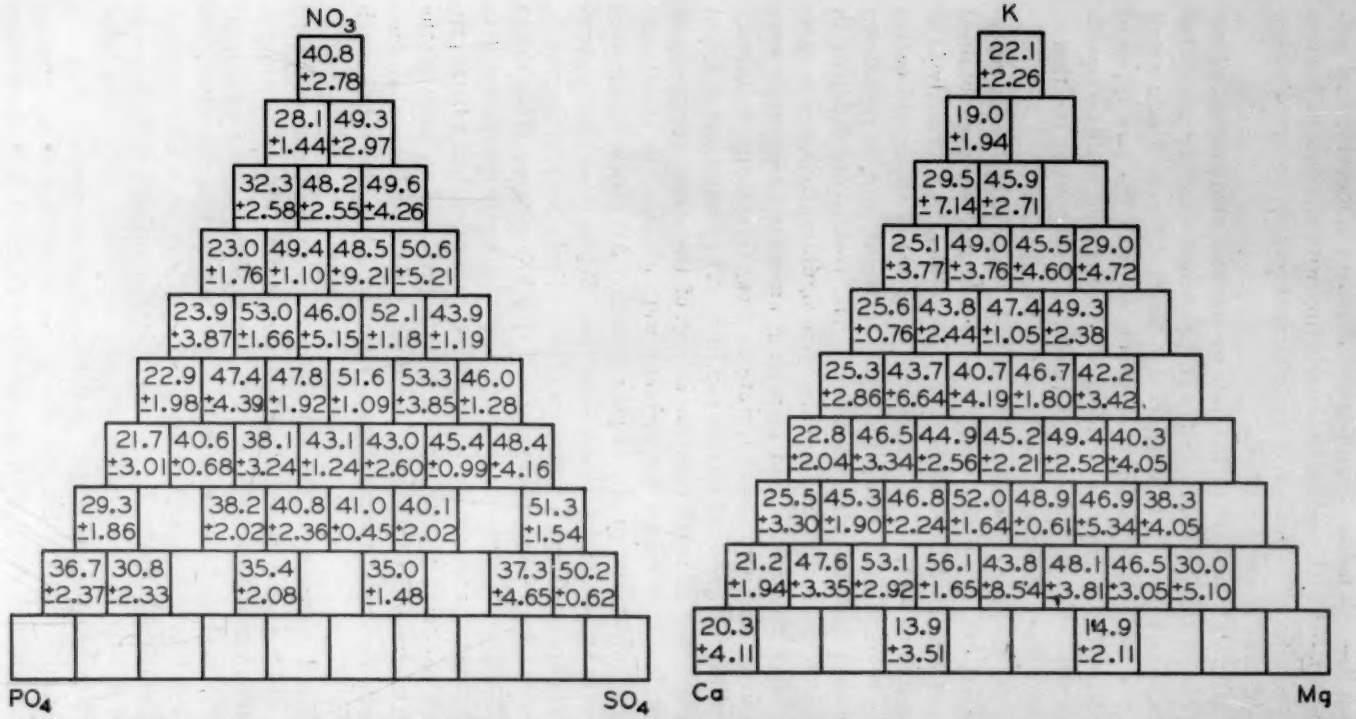

FIG. 4. Carotene values (mg./100 gm. fresh wt.) of turnip greens of sand cultures in first experiment giving treatment means with their standard error.

Anion triangle (left). The "F" values from the variance analysis are: treatments 8.82* (39 degrees of freedom); between blocks 0.87 (3 d.f.).

Cation triangle (right). The "F" values from the variance analysis are: treatments 10.98* (40 degrees of freedom); between blocks 2.48 (3 d.f.).

* Significant at the 1% level.

tively. While these differences which may be attributed to treatments and to fields are significant statistically, it seems that they have little practical importance. The differences between the plants at the laboratory and those at the two field locations are much greater than the differences between the two fields or between treatments in either field.

At each of the field locations, ten pot-cultures of each soil were established corresponding to treatment 20, table VII. These cultures were placed alongside of each field and served as a check on the effects of location. The results obtained with these cultures are given at the bottom of table VIII. The ascorbic acid values of the plants in these cultures are slightly higher than the values obtained in the field plants, but all are considerably lower than the values obtained in the pot-cultures at the laboratory. The differences between these pot-cultures and those at the laboratory indicate an appreciable effect of location or time of harvest on ascorbic acid values.

The carotene values of the plants in the pot-cultures at each field closely approximated the average values obtained in the field-grown plants. As mentioned, the differences in the carotene values between the two fields were statistically significant, and both fields gave values considerably higher than those obtained in the pot-cultures and sand cultures at the laboratory. As was the case with the ascorbic acid values, it seems apparent that variations in carotene may be correlated with time of harvest or location. In fact, all these results on carotene and ascorbic acid demonstrate that factors which were not under particular control in this experiment produced much greater variations in the vitamin content than was caused by the various treatments employed. This is particularly apparent in a comparison of the carotene and ascorbic acid values for the sand and the soil-pot-cultures, which were grown in alternating blocks at the laboratory, but analyzed on different days. As will be discussed in greater detail later, these variations in the average values obtained in the various sets of plants were apparently associated with certain differences in climate correlated with location or date of harvest.

Experiment II

Since Experiment I was carried out at a time equivalent to the spring crops, a second experiment was conducted to correspond to the fall crop, in order to amplify the results of the first. Many of the plants in the field of the first experiment sent up flower stalks, but in the second experiment they did not. The sand culture treatments were modified to include some in which the plants received nutrient solutions containing all the macro-elements, but in a greater degree of unbalance than those used previously. The plants were harvested at an earlier stage of growth than that at which they were harvested in the first.

Procedure

Seeds of the same variety were planted on August 7. The same procedures of handling the seedlings were followed as in the first experiment. The seedlings for the sand cultures were transplanted on August 26, and the

seedlings in the soil were thinned on September 4. The plants in sand cultures were started upon their respective nutrient treatments immediately after transplanting. The soil treatments were essentially the same as those of Experiment I, except that a few treatments were added to the pot-cultures and two treatments were eliminated. In most cases no more fertilizers were added to the soil. Only the nitrogen fertilizer was reapplied in original amounts. In the field the plots were cultivated lightly and the seeds planted without further treatment with the exception that a reapplication of manure was made on two of the four manured plots. In the pots containing soil additional treatments consisted of applications of various organic materials. The soil treatments are listed in table IX and the nutrient composition of the sand cultures shown in tables X and XI. The samples were collected and analyses made in a manner similar to that of Experiment I. Standard errors were computed for the data of each treatment where possible, but the analysis of variance was not made.

Results

The plants grew rapidly, especially after the transplanting and thinning dates. In general, the plants in the soil were of a greener color than were the plants in the sand cultures, although all the plants except those on deficiency treatments were of an even greenness. On September 24 (one day before samples were taken for the first vitamin analysis) careful observations were made on all of the plants. At the time of harvest, data similar to that of the first experiment were collected. Only the data of the fresh weights of the tops are included here. As in Experiment I, all observations served to demonstrate a great effect of the treatments on the growth and development of the plants, and differences in appearance associated with mineral deficiencies were similar in both experiments.

The ascorbic acid content of the plants, tables X and XI, in the sand cultures of this experiment were relatively much lower than were those of the first experiment, and the variations which might be correlated with treatment were relatively slight. Low ascorbic acid values were obtained in treatments deficient in sulfate and potassium, and high ascorbic acid values could be correlated with treatments relatively high in magnesium; i.e., 85, 86, and 87. Treatments 70, 71, and 72, which were relatively low in calcium and relatively high in magnesium, also seemed to produce high ascorbic acid values. These latter six treatments are not comparable to any of the treatments of Experiment I. In contrast to Experiment I, treatment 50, which was deficient in calcium, resulted in high ascorbic acid values. Also in contrast to Experiment I, there was no correlation between nitrogen supply and ascorbic acid content with the single exception that treatment 13, which was deficient in nitrates, produced plants relatively high in ascorbic acid. Treatments 42, 43, and 44, which received additional supplies of nitrogen in the form of ammonium nitrate, had ascorbic acid values comparable to the average values obtained in both triangles.

TABLE IX

FERTILIZER TREATMENTS APPLIED TO SOIL-POTS AND FIELD PLOTS OF EXPERIMENT II

TREATMENT	LIME PER ACRE	INORGANIC FERTILIZER PER ACRE						APPLICATION TO†				REMARKS
								POT-CULTURES		FIELD PLOTS		
		Ca	K	Mg	N	P_2O_5	S	DUNKIRK	MARDIN	DUNKIRK	MARDIN	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
1*	.....	.....	.....	.....	.....	.....	.....	+	+	–	–	
2	2	.....	.....	.....	.....	.....	.....	+	+	–	–	Ash of 10 tons of alfalfa per acre applied
3	2	.....	.....	.....	.....	.....	.....	+	+	–	–	Ash of 10 tons of alfalfa per acre applied
4*	10	.....	.....	.....	.....	.....	.....	+	+	+	+	
5*	10	.....	.....	.....	.....	.....	.....	–	–	+	+	Added microelements, residual
6*	2	.....	.....	.....	.....	.....	.....	–	–	+	+	Added microelements, residual
7*	2	29.6	43.6	13.5	20.5	.....	29.7	+	+	+	+	
8*	2	29.6	43.6	13.5	.....	.....	59.3	+	+	+	+	
9*	2	29.6	43.6	13.5	41.0	.....	.....	+	+	+	+	
10*	2	29.6	43.6	13.5	.....	105.0	29.7	+	+	+	+	
11*	2	29.6	43.6	13.5	.....	210.0	.....	+	+	+	+	
12*	2	29.6	43.6	13.5	20.5	105.0	.....	+	+	+	+	
13*	2	37.0	.....	22.5	16.4	63.6	17.8	+	+	+	+	
14*	2	.....	.....	45.0	16.4	63.6	17.8	+	+	+	+	
15*	2	74.0	.....	.....	16.4	63.6	17.8	+	+	+	+	
16*	2	37.0	72.0	.....	16.4	63.6	17.8	+	+	+	+	
17*	2	.....	144.0	.....	16.4	63.6	17.8	+	+	+	+	
18*	2	.....	72.0	22.5	16.4	63.6	17.8	+	+	+	+	

TABLE IX—*Continued*

Treatment	Lime per acre	Inorganic fertilizer per acre						Application to†				Remarks
								Pot-cultures		Field plots		
		Ca	K	Mg	N	P_2O_5	S	Dunkirk	Mardin	Dunkirk	Mardin	
	tons	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*					
19*	.....	24.7	48.0	15.0	16.4	63.6	17.8	+	+	−	−	
20*	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
21*	2	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	Added microelements, residual
22*	10	24.7	48.0	15.0	16.4	63.6	17.8	+	+	+	+	
23*	10	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	Added microelements, residual
24*	2	29.6	43.6	13.5	13.6	70.0	19.8	+	+	+	+	
25	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons alfalfa per acre applied
26	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons straw per acre applied
27*	2	.....	.....	.....	.....	.....	.....	−	−	+	+	10 tons manure per acre, residual
28*	2	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	10 tons manure per acre, residual
29	2	.....	.....	.....	.....	.....	.....	−	−	+	+	10 tons manure per acre, residual and applied
30	2	24.7	48.0	15.0	16.4	63.6	17.8	−	−	+	+	10 tons manure per acre, residual and applied
31	2	.....	.....	.....	.....	.....	.....	+	+	−	−	Soil puddled
32	2	.....	.....	.....	.....	.....	.....	+	+	−	−	10 tons straw per acre + N to equal C: N of 14: 1
33	2	.....	.....	.....	.....	.....	.....	+	−	−	−	Turnips transplanted to pots 3 weeks after germination.

* Treatments same as in Expt. I. Fertilizer treatments residual, with the exception of nitrogen, which was supplied in the same amounts as at the original application.

† The symbol + indicates that the fertilizers to the left have been added.

The carotene content of the leaves was low in treatments deficient in sulfate (treatment 3), magnesium (treatment 47), and potassium (treatment 57), which corresponds closely to the results of the first experiment. There are indications in the first experiment that treatments deficient in calcium produced plants low in carotene, but such was not the case in the second

TABLE X

COMPOSITION OF NUTRIENT SOLUTIONS, FRESH WEIGHTS, ASCORBIC ACID, AND CAROTENE CONTENTS OF PLANTS OF THE ANION TRIANGLE OF EXPERIMENT II

TREATMENT	COMPOSITION OF NUTRIENTS IN M. EQ./LITER*				FRESH WT. OF TOPS	ASCORBIC ACID (MG./100 GM.)	CAROTENE (μ/GM.)
	NO_3	H_2PO_4	SO_4	NH_4			
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*gm.*	*mg.*	μ
1	19.83	2.83	2.83	.....	52 ± 3.3	153 ± 1.9	43.7 ± 3.12
2	14.17	5.67	5.67	.....	37 ± 4.9	145 ± 3.3	37.2 ± 3.99
3	12.75	12.75	0.00	.....	12 ± 2.1	111 ± 6.6	19.7 ± 5.84
4	11.33	11.33	2.83	.....	32 ± 2.3	159 ± 2.2	38.9 ± 1.71
5	11.33	2.83	11.33	.....	32 ± 4.3	161 ± 3.4	32.5 ± 8.37
6	12.75	0.00	12.75	.....	8 ± 1.6	194 ± 4.7	56.1 ± 11.83
7	8.50	8.50	8.50	.....	21 ± 3.8	131 ± 2.0	37.2 ± 3.56
8	5.67	14.17	5.67	.....	18 ± 2.2	162 ± 3.0	42.7 ± 9.14
9	5.67	5.67	14.17	.....	18 ± 2.0	166 ± 5.1	37.0 ± 7.27
10	2.83	19.83	2.83	.....	10 ± 1.6	163 ± 2.1	21.9 ± 1.48
11	2.83	11.33	11.33	.....	9 ± 1.6	152 ± 2.1	34.2 ± 5.70
12	2.83	2.83	19.83	.....	9 ± 1.4	162 ± 1.2	.....
13	0.00	12.75	12.75	.....	1 ± 0.3	195 ± 4.3	34.7 ± 7.56
14	16.04	8.02	1.42	.....	36 ± 3.2	162 ± 3.6	21.4 ± 4.89
15	16.52	8.26	0.71	.....	38 ± 3.8	148 ± 2.3	44.2 ± 8.98
16	16.76	8.38	0.35	.....	31 ± 3.1	135 ± 1.4	40.5 ± 3.52
17	8.02	16.04	1.42	.....	18 ± 9.6	155 ± 4.0	24.8 ± 8.29
18	8.26	16.52	0.71	.....	26 ± 2.3	153 ± 2.4	16.6 ± 6.76
19	8.38	16.76	0.35	.....	22 ± 3.0	140 ± 2.8	31.8 ± 3.94
20	8.02	1.42	16.04	.....	25 ± 9.8	157 ± 4.1	30.2 ± 0.00
21	8.26	0.71	16.52	.....	23 ± 2.8	160 ± 4.4	40.5 ± 7.69
22	8.38	0.35	16.76	.....	21 ± 2.7	150 ± 8.3	40.0 ± 3.56
23	16.04	1.42	8.02	.....	36 ± 2.2	157 ± 1.6	42.3 ± 0.79
24	16.52	0.71	8.26	.....	42 ± 3.4	165 ± 2.7	42.7 ± 7.72
25	16.76	0.35	8.36	.....	28 ± 2.3	174 ± 3.6	42.5 ± 5.78
26	24.08	0.71	0.71	.....	39 ± 5.9	151 ± 2.1	40.6 ± 5.34
27	21.96	2.83	0.71	.....	49 ± 2.4	158 ± 2.3	36.9 ± 4.70
28	21.96	1.77	1.77	.....	48 ± 10.1	145 ± 2.6	41.3 ± 4.45
29	21.96	0.71	2.83	.....	40 ± 3.2	165 ± 2.6	44.9 ± 3.95
30	0.71	24.08	0.71	.....	3 ± 0.7	150 ± 3.4	28.6 ± 5.34
31	2.83	21.96	0.71	.....	8 ± 0.7	154 ± 3.1	32.6 ± 4.14
32	1.77	21.96	1.77	.....	3 ± 1.0	157 ± 1.8	39.9 ± 7.86
33	0.71	21.96	2.83	.....	3 ± 0.3	117 ± 0.5	42.4 ± 9.25
34	0.71	0.71	24.08	.....	3 ± 0.3	165 ± 2.0	19.9 ± 4.47
35	2.83	0.71	21.96	.....	12 ± 1.5	165 ± 2.3	27.2 ± 2.13
36	1.77	1.77	21.96	.....	6 ± 0.7	175 ± 1.3	33.4 ± 5.32
37	0.71	2.83	21.96	.....	4 ± 0.5	161 ± 9.5	18.9 ± 0.87
38	8.50	8.50	8.50	.....	29 ± 3.0	159 ± 3.3	25.9 ± 5.10
39	1.42	12.04	12.04	.....	6 ± 1.2	166 ± 6.3	37.4 ± 3.90
40	0.71	12.39	12.39	.....	3 ± 0.5	164 ± 5.7	19.1 ± 2.72
41	0.35	12.57	12.57	.....	2 ± 0.3	150 ± 3.7	31.5 ± 9.71
42	12.75	8.50	8.50	4.25	59 ± 4.6	152 ± 1.9	31.8 ± 3.85
43	17.00	8.50	8.50	8.50	77 ± 4.8	169 ± 2.5	37.9 ± 5.76
44	21.25	8.50	8.50	12.75	69 ± 15.8	151 ± 2.2	48.6 ± 13.78

* Each of these nutrient solutions contained the following milliequivalents per liter of cations: Ca = 12.0, K = 4.5, and Mg = 9.0.

experiment. Treatments 60 and 63 in which only 0.35 milliequivalents of magnesium were used also produced plants low in carotene. It may be noted that the average carotene values were very near the same in both experiments.

TABLE XI

COMPOSITION OF NUTRIENT SOLUTIONS, FRESH WEIGHTS, ASCORBIC ACID, AND CAROTENE CONTENTS OF PLANTS OF THE CATION TRIANGLE OF EXPERIMENT II

TREATMENT	COMPOSITION OF NUTRIENTS IN M. EQ./LITER*			FRESH WT. OF TOPS	ASCORBIC ACID (MG./100 GM.)	CAROTENE (μ/GM.)
	K	Ca	Mg			
	m. eq.	*m. eq.*	*m. eq.*	*gm.*	*mg.*	μ
45	19.83	2.83	2.83	28 ± 2.1	134 ± 2.1	32.5 ± 2.2
46	14.17	5.67	5.67	26 ± 5.5	137 ± 2.7	33.6 ± 6.7
47	12.75	12.75	0.00	17 ± 1.9	174 ± 3.8	23.5 ± 2.2
48	11.33	11.33	2.83	34 ± 3.6	163 ± 3.3	35.3 ± 2.6
49	11.33	2.83	11.33	34 ± 2.6	152 ± 4.3	35.5 ± 5.0
50	12.75	0.00	12.75	0.3 ± 0.27	212 ± 8.3	32.3 ± 14.6
51	8.50	8.50	8.50	37 ± 2.4	145 ± 3.1	37.4 ± 0.0
52	5.67	14.17	5.67	39 ± 4.3	150 ± 2.3	27.2 ± 4.8
53	5.67	5.67	14.17	30 ± 3.6	144 ± 3.7	40.7 ± 4.3
54	2.83	19.83	2.83	32 ± 3.4	158 ± 2.5	43.9 ± 2.2
55	2.83	11.33	11.33	33 ± 3.4	162 ± 3.5	42.9 ± 5.2
56	2.83	2.83	19.83	14 ± 3.9	156 ± 4.4	32.4 ± 6.0
57	0.00	12.75	12.75	6 ± 1.3	87 ± 3.2	23.5 ± 3.4
58	16.04	8.02	1.42	31 ± 4.0	146 ± 2.6	39.4 ± 5.0
59	16.52	8.26	0.71	33 ± 2.9	144 ± 2.8	31.5 ± 0.7
60	16.76	8.38	0.35	30 ± 0.9	140 ± 2.6	25.4 ± 6.5
61	8.02	16.04	1.42	37 ± 2.8	158 ± 2.8	40.9 ± 4.3
62	8.26	16.52	0.71	39 ± 5.7	161 ± 3.6	33.5 ± 3.3
63	8.38	16.76	0.35	35 ± 4.4	161 ± 5.9	25.7 ± 5.0
64	1.42	8.02	16.04	18 ± 2.4	174 ± 3.9	45.3 ± 6.0
65	0.71	8.26	16.52	19 ± 1.8	162 ± 1.3	45.0 ± 3.3
66	0.35	8.38	16.76	14 ± 2.4	125 ± 2.2	33.6 ± 8.1
67	1.42	16.04	8.02	30 ± 3.6	173 ± 3.3	25.1 ± 8.9
68	0.71	16.52	8.26	24 ± 2.5	136 ± 2.7	45.0 ± 3.4
69	0.35	16.76	8.38	18 ± 1.8	138 ± 3.5	36.9 ± 6.1
70	8.02	1.42	16.04	3 ± 0.8	166 ± 8.7	21.1 ± 1.0
71	8.26	0.71	16.52	4 ± 2.3	194 ± 7.9	33.0 ± 0.0
72	8.38	0.35	16.76	2 ± 1.2	188 ± 2.4	14.2 ± 3.0
73	16.04	1.42	8.02	19 ± 2.3	139 ± 2.2	33.1 ± 6.5
74	16.52	0.71	8.26	12 ± 3.3	145 ± 3.6	39.5 ± 7.4
75	16.76	0.35	8.36	3 ± 0.4	157 ± 3.6	27.7 ± 7.3
76	24.08	0.71	0.71	19 ± 2.5	123 ± 3.5	27.7 ± 4.0
77	21.96	2.83	0.71	37 ± 1.2	126 ± 2.7	30.3 ± 1.0
78	21.96	1.77	1.77	35 ± 6.8	133 ± 3.5	21.3 ± 4.3
79	21.96	0.71	2.83	18 ± 3.1	125 ± 2.6	29.2 ± 5.9
80	0.71	24.08	0.71	19 ± 1.7	151 ± 3.1	36.8 ± 1.8
81	2.83	21.96	0.71	29 ± 5.5	182 ± 2.8	40.7 ± 2.8
82	1.77	21.96	1.77	26 ± 1.7	177 ± 2.9	42.4 ± 0.7
83	0.71	21.96	2.83	24 ± 3.3	160 ± 5.1	47.9 ± 5.5
84	0.71	0.71	24.08	0.5 ± 0.51	.....	.....
85	2.83	0.71	21.96	8 ± 6.5	214 ± 150.9	.....
86	1.77	1.77	21.96	1 ± 0.3	282 ± 4.1	55.2 ± 0.0
87	0.71	2.83	21.96	4 ± 1.4	243 ± 5.5	31.1 ± 6.8

* Each of these nutrient solutions contained the following milliequivalents per liter of anions: $NO_3 = 12.0$, $H_2PO_4 = 4.5$, and $SO_4 = 9.0$.

Treatments 1, 42, 43, and 44 in which the largest amounts of nitrogen were used were richer in carotene than average; it seems apparent that in both experiments carotene content could be directly correlated with nitrogen sup-

ply. It was also evident that treatments deficient in or relatively low in phosphates produced plants high in carotene. The chlorophyll values were somewhat correlated with the carotene values, although the results are rather variable and have not been included. The chlorophyll content seemed to be relatively high in plants given treatments deficient in phosphate or rich in nitrogen.

Comparisons may be made between the amount of growth of the tops as measured by fresh weight and the vitamin values. There was almost a direct correlation between the growth in the anion triangle and the amount of nitrogen supplied, although treatments 3 and 6, which were deficient in sulfate and phosphate, respectively, showed little growth. The amount of growth varied from 1 to 29 grams per plant. In the cation triangle, decreases in growth could be correlated with amounts of potassium of 0.71 milliequivalents or less and with amounts of calcium of 1.42 milliequivalents or less. Amounts of magnesium as low as 0.35 milliequivalents resulted in nearly the optimum amount of growth, although growth was decreased appreciably when magnesium was not present in the nutrient solution. Neither the percentage of ascorbic acid nor carotene showed any close correlation with the amount of growth produced. For example, plants from treatments deficient in phosphorus were as high or higher in both ascorbic acid and carotene than plants from most of the other treatments, although very little growth was obtained. While the nitrogen supply produced greater effects on growth than any other nutrient available, there was little correlation between nitrogen supply and ascorbic acid content, and the carotene content varied but slightly. Treatments 42, 43, and 44, which produced by far the greatest amount of top growth of any of the treatments, had average ascorbic acid values, and the carotene values were only slightly above average.

In the soil treatment the response to treatment was less marked in terms of growth (table XII) in this experiment than in the spring crop, probably because most of the fertilizer treatments were not repeated. The treatments where organic material or ash was applied, however, produced exceptionally good growth.

The only effect of treatment on vitamin content was in treatment 34 (alfalfa incorporated) where an increase in carotene[3] was accompanied by a decrease in ascorbic acid.[4] The data from the sand-culture experiments would not indicate that this effect was due to nitrogen supply.

The ascorbic acid values for the plants grown in soil-pot-cultures were lower than in the first experiment (cf. sand cultures) while the values for plants grown at the fields were higher than in Experiment I. In the second experiment, the ascorbic acid content of the field plants was higher than those of the soil-pot-cultures which were also higher than those for the sand cultures. The carotene values for the second experiment, however, are

[3] Significant at 5 per cent. level in a "T" test.

[4] Significant at 5 per cent. level in a "T" test.

TABLE XII

FRESH WEIGHT, CAROTENE CONTENT, AND ASCORBIC ACID CONTENT OF TURNIPS GROWN IN SOIL, EXPERIMENT II

Treatment	Fresh weight of tops (gm./plant) Pot-cultures Dunkirk	Fresh weight of tops (gm./plant) Pot-cultures Mardin	Fresh weight of tops (kg./9′ row) Field plots Dunkirk	Fresh weight of tops (kg./9′ row) Field plots Mardin	Carotene (μ/100 gm. fresh wt. of leaves) Pot-cultures Dunkirk	Carotene (μ/100 gm. fresh wt. of leaves) Pot-cultures Mardin	Carotene (μ/100 gm. fresh wt. of leaves) Field plots Dunkirk	Carotene (μ/100 gm. fresh wt. of leaves) Field plots Mardin	Ascorbic acid (mg./100 gm. fresh wt. of leaves) Pot-cultures Dunkirk	Ascorbic acid (mg./100 gm. fresh wt. of leaves) Pot-cultures Mardin	Ascorbic acid (mg./100 gm. fresh wt. of leaves) Field plots Dunkirk	Ascorbic acid (mg./100 gm. fresh wt. of leaves) Field plots Mardin
	gm.	*gm.*	*kg.*	*kg.*	μ	μ	μ	μ	*mg.*	*mg.*	*mg.*	*mg.*
1	6.3	1.0	.....	.....	.....	.....	.....	.....	185 ± 5.3	168 ± 14.7	.....	.....
2	9.6	5.2	0.46	0.12	45.1 ± 5.00	31.7 ± 5.90	37.8 ± 1.59	40.2 ± 5.38	177 ± 7.7	187 ± 8.6	202 ± 10.6	234 ± 9.5
3	5.7	7.3	.....	.....	.....	.....	.....	.....	170 ± 5.9	172 ± 10.4	.....	.....
4	10.0	10.3	0.28	0.16	55.0 ± 1.37	29.7 ± 7.85	.....	.....	178 ± 4.2	198 ± 27.9	222 ± 7.1	203 ± 5.4
5	.....	.....	0.56	0.24	.....	.....	.....	.....	.....	.....	192 ± 7.2	218 ± 22.3
6	.....	.....	0.42	0.21	.....	.....	.....	.....	.....	.....	226 ± 9.9	216 ± 10.9
7	7.4	7.5	0.52	0.24	.....	.....	.....	.....	180 ± 7.4	206 ± 5.3	218 ± 7.8	222 ± 18.5
8	6.7	5.5	0.34	0.20	.....	.....	.....	.....	178 ± 7.9	178 ± 10.1	207 ± 14.6	206 ± 13.8
9	11.0	10.4	0.41	0.27	.....	.....	.....	.....	178 ± 10.9	170 ± 8.9	190 ± 6.4	233 ± 8.9
10	8.9	6.3	0.50	0.28	.....	.....	.....	.....	186 ± 11.4	214 ± 11.7	200 ± 7.3	209 ± 6.7
11	10.4	5.5	0.88	0.18	.....	.....	.....	.....	185 ± 9.0	182 ± 4.2	191 ± 8.3	237 ± 17.8
12	10.6	10.3	0.70	0.37	.....	.....	.....	.....	191 ± 8.8	191 ± 4.0	216 ± 15.0	204 ± 10.4
13	8.9	6.8	0.57	0.31	.....	.....	.....	.....	162 ± 12.6	196 ± 10.8	196 ± 5.7	214 ± 14.6
14	9.4	7.9	0.85	0.21	.....	.....	.....	.....	185 ± 8.8	213 ± 10.3	181 ± 6.7	235 ± 13.5
15	10.1	6.1	0.54	0.25	.....	.....	49.2 ± 2.12	45.8 ± 2.63	179 ± 5.0	195 ± 10.2	200 ± 6.7	236 ± 8.1
16	7.2	6.7	0.42	0.32	.....	.....	.....	.....	170 ± 4.7	212 ± 8.8	203 ± 10.7	187 ± 7.2
17	10.8	6.5	0.64	0.45	.....	.....	.....	.....	181 ± 3.6	152 ± 18.0	199 ± 9.1	214 ± 11.9
18	10.4	9.1	0.56	0.33	.....	.....	.....	.....	175 ± 6.5	190 ± 9.4	203 ± 7.1	200 ± 8.5
19	6.5	2.6	.....	.....	.....	.....	.....	.....	180 ± 6.7	208 ± 24.6	.....	.....
20	10.8	8.2	0.47	0.26	37.2 ± 7.43	32.4 ± 6.11	39.1 ± 5.22	49.0 ± 3.80	179 ± 6.4	187 ± 7.7	206 ± 14.4	196 ± 11.1
21	11.6	8.3	0.54	0.28	29.0 ± 4.46	36.6 ± 3.05	.....	.....	190 ± 9.0	194 ± 9.2	213 ± 10.5	206 ± 9.6
22	10.0	9.5	0.66	0.33	36.0 ± 3.25	42.6 ± 5.58	.....	.....	188 ± 5.3	168 ± 6.0	215 ± 10.6	216 ± 14.0
23	.....	.....	0.70	0.33	.....	.....	36.6 ± 4.77	45.3 ± 3.03	.....	.....	202 ± 9.7	188 ± 5.4
24	8.2	6.5	0.40	0.28	41.2 ± 6.50	38.0 ± 8.66	38.6 ± 3.34	50.4 ± 0.56	164 ± 7.4	182 ± 7.7	206 ± 5.7	238 ± 7.9
25	12.7	22.8	.....	.....	.....	.....	.....	.....	169 ± 2.6	190 ± 7.4	.....	.....
26	16.3	28.9	.....	.....	33.3 ± 4.02	40.0 ± 3.37	.....	.....	182 ± 7.6	188 ± 11.9	.....	.....
27	.....	.....	0.75	0.32	.....	.....	.....	.....	.....	.....	200 ± 11.6	202 ± 11.3
28	.....	.....	0.82	0.51	.....	.....	.....	.....	.....	.....	203 ± 12.9	203 ± 3.9
29	.....	.....	1.57	0.73	.....	.....	42.1 ± 5.04	46.0 ± 2.66	.....	.....	160 ± 4.5	183 ± 9.3
30	.....	.....	1.80	0.74	.....	.....	.....	.....	.....	.....	155 ± 7.5	184 ± 12.4
31	13.2	16.1	.....	.....	33.2 ± 4.00	49.7 ± 5.39	.....	.....	182 ± 6.9	168 ± 8.0	.....	.....
32	26.1	23.6	.....	.....	.....	.....	.....	.....	170 ± 12.7	156 ± 8.4	.....	.....
33	20.1	.....	.....	.....	.....	.....	.....	.....	183 ± 11.0	.....	.....	.....
34	28.4	32.8	.....	.....	64.3 ± 4.10	64.0 ± 16.25	.....	.....	144 ± 2.5	142 ± 5.6	.....	.....
	Average vitamin values				41.6	40.5	40.6	46.1	177	185	200	211
20	Dunkirk soil in pots at fields				.....	.....	39.7 ± 3.60	51.5 ± 8.06	.....	.....	211 ± 6.3	227 ± 5.6
20	Mardin soil in pots at fields				.....	.....	19.9 ± 3.46	36.9 ± 5.30	.....	.....	221 ± 8.6	221 ± 4.5

essentially the same for plants grown in field plots, soil-pot-cultures, and sand cultures.

Discussion

With respect to the ascorbic acid content of turnip greens, both of these experiments indicate that the differences in environment of one location and another or from one date to another have a marked effect upon the ascorbic acid content. Just which factors were responsible for the variations which occurred between locations in these experiments is unknown. In the original organization of the work, emphasis was placed upon the expected variations in ascorbic acid between treatments, and it seemed logical to analyze all of the plants from one location on the same day, and therefore no separation of the effects of location and date of harvest is possible. In certain of the data, it is possible to determine which differences were associated with date of harvest, and while these are statistically significant, they are of relatively less magnitude than the differences between locations.

The plants grown in soil showed no indication that fertilizer treatment influenced ascorbic acid content to any great extent, in spite of the fact that the fertilizer treatments employed had a marked effect upon the growth of the plants. In the sand cultures, significant effects of treatments on vitamin content were produced in certain cases where the treatments were deficient in one or more nutrient elements, but in all these cases very little growth of the plants was obtained. Increasing nitrogen supplies seemed to result in increasing ascorbic acid values in the first experiment, while in the second no appreciable effect of nitrogen supply was noted. In the first experiment, conducted in the early summer, the average ascorbic acid values were much higher than in the second. In the preliminary experiments with plants produced in the greenhouse during the winter, the average ascorbic acid values were approximately half those obtained in the experiments conducted during the summer. The growth of the plants during the winter months was not as rapid as during the summer, but at the time the plants were harvested in the preliminary work they were of about the same general appearance as those harvested during the summer. Thus, the average ascorbic acid values obtained in experiments at various seasons differed greatly.

In a review of the literature, MAYNARD and BEESON (**10**) found considerable disagreement among the various investigators as to whether or not fertilizer treatments affected the ascorbic acid content of different varieties of plants. Many investigators claimed no effect of fertilizer treatment; others claimed that nitrogen fertilization increased ascorbic acid content, and still others that nitrogen fertilization decreased it. No reports on the effects of fertilizers on ascorbic acid in turnip greens were mentioned. In a recent publication, REDER *et al.* (**15**) found an effect of nitrogen fertilization on ascorbic acid content of turnips and a significant interaction between effects of nitrogen fertilization and of location where the plants were grown. They suggested that one of the reasons for the lack of agreement among investigators as to the effect of nitrogen on ascorbic acid content may be

because of this interaction and because differences associated with location are so much greater than the effect of fertilizer. This suggestion is in agreement with the results obtained here if one assumes that factors responsible for locational effects are the same as those that cause seasonal effects (i.e., climate variables), since an effect of nitrogen supply on ascorbic acid content (in sand cultures) was noted at one season of the year and not at another. REDER *et al.* found a consistent decrease in the ascorbic acid content of greens which received potassium fertilization, and they point out that this effect of potassium fertilization is not confirmed by the work of several other investigators with other plants. They did find a significant interaction of potassium effect and location indicating that potassium fertilization reduced the ascorbic acid content more sharply at one location than at others. In the work reported here, no significant effect of potassium fertilization of soils, nor of potassium supply to the plants in sand culture was noted with the exception that in sand cultures those treatments which were deficient in potassium produced plants which were low in ascorbic acid. This is in contrast to the above mentioned work of REDER *et al.* in that in our work a deficient supply of potassium resulted in a decrease in ascorbic acid instead of an increase.

A further comparison between the work presented here and that of REDER *et al.* seems worth while. In the work of the latter investigators, higher ascorbic acid values were obtained in the fall than in the spring, which is in contrast to our results. The marked effect of environmental factors other than soil variables is apparent in both series of investigations. REDER *et al.* found the lowest ascorbic acid values at the location with the highest average daily rainfall, and pointed out in comparing the average values at several locations, that there seemed to be an inverse relationship between the amount of rainfall and ascorbic acid content, and a direct relationship between the amount of sunshine and ascorbic acid. We have not been able to correlate the major variations of ascorbic acid which were obtained in this work with any particular environmental factor or factors. It seems apparent, however, that some factor or factors not under control or under observation in this work produced far greater effects on ascorbic acid content than were produced by any of the treatments used, with the possible exception of certain deficiency treatments in the sand cultures in which so little growth was produced that the plants doubtless would have had little commercial value as "greens."

MAYNARD and BEESON (**10**) state, "Studies that reveal any very useful data on the effect of fertilization or other cultural practices that might modify the carotene content of the plant are few because most of the reported findings are complicated by questions of varieties and as to the control of the many other factors of the plant's external environment." They discuss several investigations which indicate no effect of fertilization on the carotene content and a few which indicate some effect of fertilization. In their review, they cited some investigations which indicated an effect of

seasonal conditions and climatic environmental factors on carotene content. In the work reported here, little effect of fertilization was noted in plants growing in the soil. In sand cultures, there was fair agreement between the two experiments which indicated that deficiencies of sulfur, nitrogen, and potassium caused a decrease in carotene content, while a deficiency of phosphorus resulted in no decrease. Perhaps the most striking result of this work is the indication that any treatment which resulted in visible chlorosis resulted in appreciable decreases in carotene. This was further supported by the evidence of some correlation between chlorophyll and carotene content in the second experiment. No consistent correlation between the amount of growth and carotene content was found. The turnip plants used in the preliminary studies which were grown in the greenhouse during the winter time had much higher carotene values than any of those produced during the summer (the reverse relationship was found for ascorbic acid). There were differences in carotene content between the two experiments and differences between one location and another in each experiment indicating that environmental factors associated with climate are of considerable importance in determining carotene values.

It would appear that those climatic factors which lead to low ascorbic acid values tend to result in high carotene values since in the majority of the data, seasonal conditions and location factors correlated with high ascorbic acid values were also correlated with low carotene values and vice versa.

Since there was very little difference in the ascorbic acid or carotene values of the plants in one soil as compared with those in another soil when the soils were contained in pots, it seems unlikely that the locational differences noted in these experiments are due to soil conditions. That some of the variations might have been due to the particular date of harvest serves further to indicate the possibility that the environmental factors, such as light, temperature, humidity, etc., are of great importance in determining vitamin values. As a result of the work recorded here, a series of experiments with turnips has been carried out in chambers in which most of the environmental factors were under careful control. This work which is reported in another paper (**8**) has indicated that light intensity is of very great importance in determining vitamin content of turnip greens, and consideration of the data contained here certainly does not contradict such a conclusion. Variations such as occur between one location and another or between one harvest date and another might well be related to the light intensity prevailing just previous to harvest in each case. The significant conclusion that may be made from the experiments reported here would seem to be that variations in the supply of macronutrient elements in sand cultures and variations in fertilizer practices in soils seem to have little effect on the carotene and ascorbic acid content of the plants. Variations associated with location in which the plants are grown or season at which the plants are harvested seem to be of much greater magnitude.

Summary

1. Shogoin turnips were grown in sand cultures supplied with varying amounts of macronutrient elements to make a total of 87 treatments, in soil-pot-cultures, and soil field plots with 26 fertilizer treatments. Two complete experiments were conducted; one in the late spring and early summer, the other in the early fall.

2. At the time of harvest, ascorbic acid and carotene analyses were made. In addition, data were taken on the number of leaves, the total leaf length, fresh, and dry weights of roots and tops, length and diameter of the enlarged portion of the tap root, as well as detailed visual observations. The growth data were used as an indication of the responses of the plants to treatment, and an attempt was made to correlate these with the ascorbic acid and carotene values. The data for the first experiment were analyzed by statistical methods.

3. Preliminary analyses for ascorbic acid and carotene were made to determine the best methods of sampling and analyzing for these constituents.

4. With respect to the ascorbic acid content of turnip greens, both of these experiments indicate that the differences in environment between locations or between dates have a marked effect. Differences between the values obtained in the two experiments conducted at two different seasons were also relatively great. Only minor differences in ascorbic acid could be associated with variations in the supply of macronutrient elements to plants grown in sand culture except in complete deficiencies of some macronutrients. No differences were associated with fertilizer treatment in soil-pot-cultures or in field plots, in spite of the fact that variations in growth and appearance of the plants were relatively great in the different treatments.

5. The carotene content of the plants grown in the separate experiments varied greatly, indicating a marked influence of season. Variations in carotene content of plants grown in different locations in any given experiment were also great. No appreciable effect of fertilizer treatment on the carotene content of plants grown in soil was noted. In sand cultures, deficiencies of sulfur, nitrogen, and potassium caused a decrease in carotene content while a deficiency of phosphorus resulted in no decrease. Any treatment which resulted in visible chlorosis resulted in appreciable decreases in carotene. No consistent correlation between the amount of growth and the carotene content was found.

6. Both the ascorbic acid and carotene contents seem to be primarily influenced by environmental variables associated with season and location, and there was some evidence that those particular conditions which led to a high ascorbic acid content resulted in a low carotene content and vice versa.

7. A discussion is given of the possible significance of this work with the suggestion that light intensity may be the environmental factor playing the dominant rôle in determining ascorbic acid values.

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DROUGHT RESISTANCE IN RANGE AND PASTURE GRASSES[1]

ODELL JULANDER

(WITH EIGHT FIGURES)

Introduction

Rainfall is the limiting factor in the success of the livestock industry on the western range. Precipitation is especially critical in the southern Great Plains, the Southwest, and the semi-desert areas of the Intermountain Region. Here drought and its accompanying overgrazing constitute the major range problems and have been responsible for serious depletion of the forage resources of the region.

NELSON (**29**) has shown that in years of drought basal area of black grama may fall as low as 10 or 11 per cent. of the maximum stand and 20 per cent. of a 13-year average. CRADDOCK and FORSLING (**8**) found in Southern Idaho that the volume of forage produced varied from 41 above to 33 per cent. below the nine-year average, largely as a result of winter and spring precipitation. Drought and overgrazing together result in much greater reduction than does drought alone or drought and moderate grazing (**46, 47**). STEWART (**43**) reported that Forest Service studies on western Utah winter ranges show that the drought from 1931 to 1934 caused a 20 per cent. decrease in available forage plants on ungrazed plots, but on overgrazed areas, depletion was approximately 60 per cent. SAVAGE (**36**) and SAVAGE and JACOBSON (**37**), studying drought injury in the central and southern Great Plains, reported that as grazing was intensified all grasses decreased in all areas where drought was severe. LANTOW and FLORY (**17**), working on semi-desert grassland of New Mexico, concluded that permanent injury may be caused by drought on overgrazed range, but that on properly grazed range the recovery of the grass stand with increased precipitation is rapid. U. S. Forest Service workers (**6, 7, 29, 33**) have continuously stressed the injurious effect of drought on range plants. BOUGHMAN (**3**) showed that the root growth of several different range grasses is inhibited by clipping, the degree varying directly with the amount of clipping. SILKER (**41**) found that drought survival of western wheat grass seedlings depended upon the formation of rhizomes.

Most workers agree that the physiological bases for drought and frost resistance are similar. There is disagreement, however, in the theories of the mechanism of resistance. Several workers (**5, 10, 19, 20, 30, 34**) have emphasized the importance of bound or "unfreezable" water in drought and frost resistance. This idea is questioned by other investigators (**40, 49**). PALTRIDGE and MAIR (**32**) concluded that xerophytes had a high water balance, but later work along this line by BAILEY (**1**) and KLOMP (**16**) failed to show a correlation between water balance and the xerophytic nature of

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the natural habitat of the plants. High concentration of cell sap is often associated with drought resistance. LEBEDINCEV (**18**) stated that the main rôle in determining the water retaining capacity of plant cells was played by osmotic substances and not by colloids. Other workers (**34, 42, 44**) have stressed the importance of hydrophyllic colloids in this rôle.

LOOMIS (**22**) has shown that food reserves are necessary for differentiation processes such as occur in plants during hardening. It is generally agreed (**21, 26, 27, 44**) that carbohydrate reserves are essential to the development of frost resistance in plants. Several investigators in this field (**34, 42, 44**) believe that carbohydrates are also necessary for drought resistance since the hardening process is essentially the same. Other workers (**4, 26, 27, 35, 48**) have shown the relationship between grazing and food reserves. Several workers (**11, 18, 21, 31, 38, 39, 44**) have investigated the changes taking place in plants during the hardening process. MAXIMOV (**24, 25**), in 1929, made a comprehensive review of the basis of frost and drought resistance up to that date. SCARTH, LEVITT, and SIMINOVITCH (**21, 38, 39**) have done considerable detailed research on the nature of cold and drought resistance in plants. They found that hardened protoplasm is less susceptible to coagulation or rupture during drought, is more permeable, has a lower viscosity, is more colloidal, and has a greater water-binding capacity than does unhardened protoplasm.

The emphasis in past work has been on resistance to dehydration with little attention to the independent or interacting effects of temperature. High temperatures normally accompany periods of drought, and, with the removal of the protecting cover by overgrazing, lethal soil temperatures become a threat to the survival of the grass roots and rhizomes. HEILBRONN (**12**), in 1924, and HEILBRUNN (**13**), in 1928, made detailed studies on the effect of high temperatures on living protoplasm. BELEHRADEK (**2**), in 1935, made a very comprehensive review of work done up to that date on temperature and living matter. He pointed out that heat injury is so complex that it cannot be explained by any one theory. He also called attention to the fact that the time factor in heat action is important, a fact which has been overlooked by many workers. SAVAGE (**36, 37**) and MUHLER and WEAVER (**28**) have recognized the damaging effect of high temperatures on range grasses.

Much time has been spent in studies of drought resistance in forage plants, and several attempts have been made to develop a simple and reliable method of testing the ability of these plants to withstand arid conditions. Some progress has been made, but no substitute for field reactions under drought conditions has been developed. Our work has been directed toward the solution of the problems of temperature injury and the use of resistance to high temperature as an index of drought resistance. Our purposes have been: (a) to test the heat resistance of various range grasses as a factor in drought resistance; (b) to determine the effect of carbohydrate supply and protoplasmic differentiation on the ability of plants to withstand heat; and

(c) to investigate the relationship of overgrazing to plant food reserves, soil temperatures, and drought resistance.

Methods

Studies of the effect of clipping and drought on heat resistance

Grasses with natural habitats differing in moisture and temperature conditions were selected for study. The following species were used: (a) Bermuda grass [*Cynodon dactylon* (L.) Pers.] which thrives in the hot climate of the South; (b) buffalo grass [*Buchloe dactyloides* (Nutt.) Engelm.] which has the reputation of being very drought resistant on the Great Plains; (c) bluestem or western wheatgrass (*Agropyron smithii* Rydb.) which has a wide distribution on the western range on arid and semi-arid areas; and (d) slender wheatgrass [*Agropyron pauciflorum* (Schwein.) Hitchc.] which grows chiefly in the foothills and mountains of the western range.

Grasses were transferred from field conditions to greenhouse ground plots where they were subjected to different treatments for a period of two months. Drought conditions were maintained on one series of plots while the other series was kept well watered. Plants on the south half of each plot were clipped bi-weekly to a 2-inch height. This procedure provided the following treatments for each species: (a) watered and clipped; (b) watered and unclipped; (c) dry and clipped; and (d) dry and unclipped. This procedure was used in an attempt to produce plants of four different food reserve levels ranging from very low in the first treatment to very high in the fourth treatment. The plants subjected to drought would presumably be hardened while the well-watered plants would be unhardened. The same treatments were applied to bluegrass in bench flats in the greenhouse. The same soil mixture was used, and flats for the various treatments were located at random.

Following drought and clipping treatments, plant samples were taken from each plot at mechanically spaced intervals and subjected to heat treatment. Individual plants were as uniform in size as possible. The rhizomes or stolons were cut to 1½-inch lengths. Roots were left intact and kept moist between wet paper towels. Lots of eight plants were used for each treatment, and each treatment was run in duplicate. The plants were placed in glass tubes, which were stoppered with cotton-covered cork, and immersed in a constant temperature water bath. Control samples were placed in similar tubes and kept at room temperature. The water bath was held at $48 \pm 0.1°$ C. (a temperature indicated by preliminary tests) and treatments of 0, ½, 1, 2, 4, 8, and 16 hours time were given to each species.

After the heat treatment, the plants were transplanted immediately to an outside bed, watered well, and permitted to grow for a period of approximately four weeks. At the end of this period the recovery of the individual plants in each sample was judged according to a previously arranged rating. Plants of excellent vigor were given a rating of ten, those of moderate vigor

six, those of poor vigor two, and the dead ones zero. The standard was based on the control samples, and vigor classes were determined by height, growth, color, and sturdiness. Average ratings were used for the figures.

Studies of food reserves of range grasses as affected by grazing and drought

To ascertain the status of the food supply of range grasses as affected by drought and grazing, and as affecting drought resistance, samples of roots, root crowns, and rhizomes (where present) were collected and analyzed. Plots in near-virgin condition with good stands of wheatgrasses were located on a sagebrush-wheatgrass range near Ogden, Utah. One area was in the undisturbed portion of North Ogden cemetery and another along a railroad right-of-way. One area was covered with a good stand of bluebunch wheatgrass [*Agropyron spicatum* (Pursh.) Scribn. and Smith] and the other with bluestem. Duplicate ten-gram root samples were taken at each location on June 14 and 15. The plants had reached approximately their maximum vegetative growth, but the moisture supply had been plentiful, and the plants had experienced no drought.

Four representative plots six feet square were selected at each of the two locations. On two of these plots, selected by lot, all vegetation was clipped to a 2-inch height. The plan called for repeated clipping through the summer on these plots, but drought conditions followed the clipping treatment and practically no new growth was made.

On August 28 to 30 of the same summer, duplicate root samples were taken on clipped and unclipped plots. Between samplings only traces of rain had fallen. The average annual precipitation for this area is around eleven inches with most of the moisture falling during the late fall, winter, and early spring. Drought conditions during the period of study were fairly typical. The grass foliage had begun to dry up about two weeks after clipping so they had been subjected to nearly two months of rather severe drought by the time the second root samples were taken. The ten-gram root samples for chemical analyses were obtained by digging twelve plants for each sample; these were taken into the laboratory where the dirt was removed by washing in cold water and the tops were clipped to a $\frac{1}{2}$-inch height. The samples were dried by blotting with paper towels. Roots, rhizomes, and stem bases were included in the samples. The twelve plants from each plot were mixed and ten-gram samples weighed out and immediately placed in jars containing 80 per cent. boiling grain alcohol and steeped for 20 minutes to stop enzymatic action and preserve the samples.

Analyses of colloidal and non-colloidal carbohydrates were made on all samples collected. Non-colloidal carbohydrates were extracted by repeated steeping in 80 per cent. alcohol and cleared with neutral lead acetate. Total sugars, sucrose, and reducing sugars were determined by the Munson-Walker-Bertrand method in accordance with procedures outlined by Loomis and Shull (**23**). The dry residue from these samples were weighed accu-

rately. They were then ground in a burr mill and the pulverizing completed in a mortar to pass a 200-mesh screen. Duplicate one-gram samples were extracted with hot water to remove soluble colloidal carbohydrates. The cleared extractions were hydrolyzed with dilute hydrochloric acid in an autoclave and determinations of glucosans and levulosans made according to procedures outlined by LOOMIS and SHULL (**23**). All calculations were converted to a green-weight basis.

STUDIES OF SOIL TEMPERATURES ON HEAVILY-GRAZED AND PROTECTED AREAS

Soil temperatures were taken at different depths on heavily-grazed and on ungrazed areas to determine the effect of vegetative cover on soil temperature. Studies were made on a semi-desert grassland type near Alamogordo, New Mexico, in early July of 1941. A protected area was selected along a railroad right-of-way which supported a good stand of grass, chiefly alkali sacaton [*Sporobolis airoides* (Torr.) Torr.]. Just through the fence a contrasting area was selected which had been heavily grazed until only about one-tenth of the original grass cover remained.

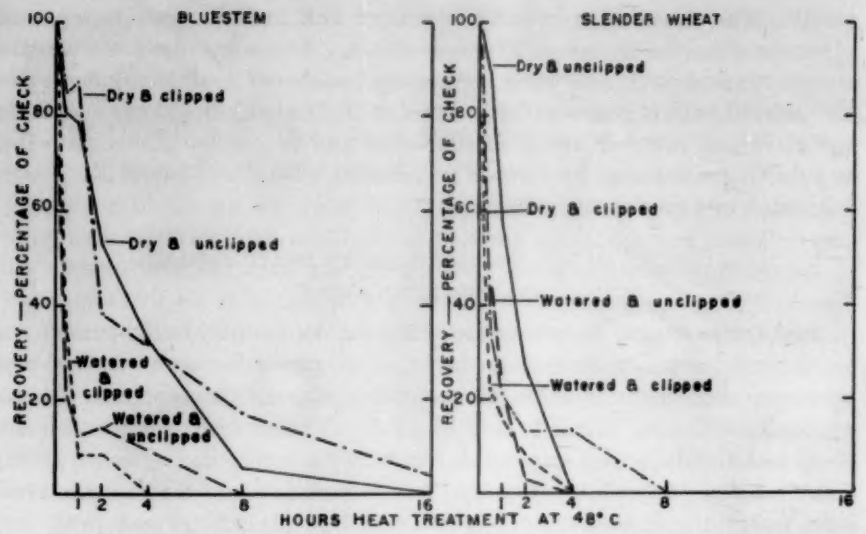
Three thermometers were placed on each area at each of the following depths: surface ($\frac{1}{2}$ of mercury above ground), $\frac{1}{2}$, 1, 2, 4, and 8 inches. The three thermometers of the same depth were located; one in the center, one 4 inches from the edge of the crown, and one half way between two typical grass clumps. Holes the same size as the thermometer were carefully made, slightly deeper than the desired depth. A little loose soil was poured in to serve as a cushion in which the bulb could be buried without breaking. The thermometers were then inserted to the desired depth. A wooden holder protected the thermometer from the direct rays of the sun. This holder was bolted to a strap iron peg which was bent to provide a 6-inch offset so the peg would not interfere with the soil adjacent to the thermometer. This offset was always placed to the south and the position of the wooden holder adjusted to shade the thermometer at mid-day without shading the soil where the thermometer was inserted.

Thermometers protected from the direct rays of the sun were located one foot above ground for air temperatures. Readings were made at hourly intervals for three consecutive days. Similar soil temperatures were taken on heavily-grazed and protected bluegrass sod at a 7,500-foot elevation in the Lincoln National Forest of New Mexico. The experiment was repeated also on the clipped and unclipped wheatgrass plots in the North Ogden, Utah, cemetery (fig. 5A) in August, 1941.

Results

RESISTANCE OF CROWNS AND RHIZOMES TO HIGH TEMPERATURES

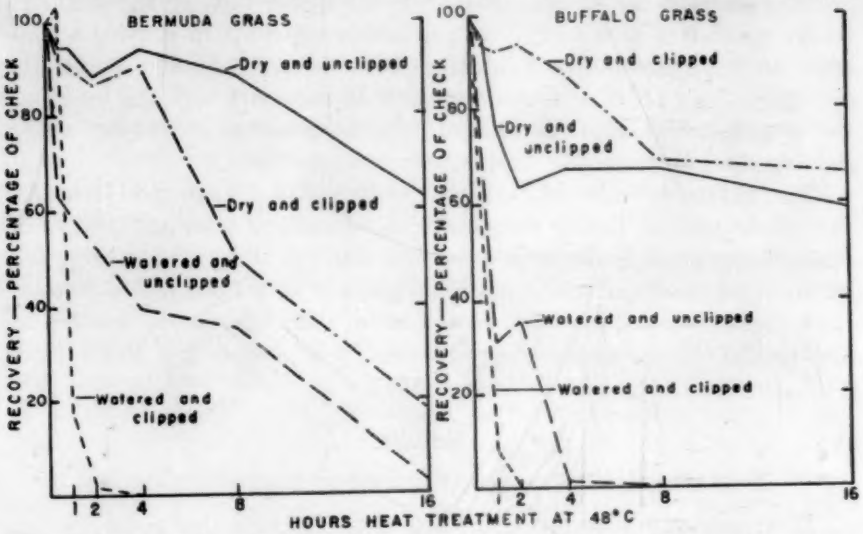
HEAT RESISTANCE OF DIFFERENT SPECIES.—The fact that some species of grass thrive naturally in areas of high temperatures while others are found only in the cooler sites, suggests a difference in their ability to withstand



FIGS. 1 and 2. Recovery of bluestem and slender wheatgrass, with varying previous treatment, after heating at 48° C.

high temperatures. The previous hardening treatments given the grasses would also be expected to influence their heat resistance. Both expectations are supported by the data of figures 1 to 4 which show the recovery of four grass species with varying hardening treatments from exposures to a temperature of 48° C.

Little difference was observed in the heat resistance of the four species in the unhardened condition. When the grasses were watered and clipped,



FIGS. 3 and 4. Recovery of Bermuda and buffalo grasses, with varying previous treatment, after heating at 48° C.

producing unhardened plants with very low food reserves, all species were killed readily. In the watered and unclipped series Bermuda grass stood out as the more heat resistant, with indications that buffalo grass in this condition was slightly more resistant than the two wheat grasses. Drought-hardened Bermuda and buffalo grasses were able to withstand considerably more heat exposure than the other two species (figs. 3, 4). Slender wheatgrass was apparently the least resistant of the four species with bluestem wheatgrass in an intermediate position.

Although moisture and clipping treatments were not randomized, the species were, and an analysis of variance of the data brings out the important comparisons (table I). According to this analysis the difference be-

TABLE I

ANALYSIS OF VARIANCE OF HEAT TREATMENT DATA

SOURCE	SUM OF SQUARES	DF	MEAN SQUARES
Total	137,100	223	
Moisture	19,389	1	19,389.00*
Species	29,730	3	9,910.00*
Moisture × species	12,823	3	4,274.33*
Clipping	2,238	1	2,238.00*
Moisture × clipping	254	1	254.00*
Species × clipping	3,173	3	1,057.67*
Moisture × species × clipping	583	3	194.00*
Treatment	51,569	6	8,594.83*
Moisture × treatment	6,302	6	1,050.34*
Species × treatment	2,570	18	142.77*
Moisture × treatment × species	2,565	18	142.51*
Clipping × treatment	217	6	36.17*
Moisture × clipping × treatment	832	6	138.67*
Species × clipping × treatment	1,470	18	81.67*
Moisture × species × clipping × treatment	2,041	18	113,39*
Error	1,341	111	.....
Replications	3	1	.....
Total error (error + replications)	1,344	112	12.00

* Significant at the 1 per cent. level.

tween species was highly significant, and species, and interactions in which species were involved, account for a high percentage of the total sum of squares.

Kentucky bluegrass (*Poa pratensis* L.), either from field plats or with greenhouse treatments similar to those used in the above experiment, was quickly killed by 48° C. and a temperature of 45° C. was used to show the greater heat resistance of previously hardened plants. Listed in order of heat resistance, Bermuda and buffalo grasses stand out as very resistant, bluestem as intermediate, slender wheatgrass and smooth brome as low, and Kentucky bluegrass as the least resistant. These results agree with field observations. In the hot regions of the Southwest bluegrass is used for lawns only under the protection of partial shade while Bermuda grass thrives in full sunlight. SAVAGE (**36**) lists buffalo grass as the most drought

resistant species on the Great Plains and states that although bluestem was mostly killed out, it withstood drought better than most tall grasses. Bluestem, while not found in the low, hot deserts of the Southwest, extends well down into the foothills. It also thrives in the semi-desert sagebrush-grass types of the Great Basin and through the Great Plains where temperatures are moderately high. Slender wheatgrass does not extend as far down the mountains into the hot, arid regions nor as far south in the Great Plains as does bluestem. It thrives best at moderate elevations in the mountains and on the northern Great Plains.

The ability of these grasses to withstand heat is apparently an important factor in determining their natural habitat. Heat resistance also appears to be an indication of the drought resistance of the grasses studied. Judging from their natural habitat they would fall in the same order when listed according to drought resistance as when listed according to their heat resistance. Kentucky bluegrass is much less drought resistant than the other species tested, followed by slender wheatgrass and smooth brome, with bluestem intermediate and buffalo grass outstanding in drought resistance. The only question is with respect to Bermuda grass which grows chiefly in areas of fairly high annual precipitation. In such areas, however, rainfall distribution is often uneven and severe drought periods are not uncommon. Bermuda grass survives these drought periods and has the reputation of being one of the most drought-resistant grasses in its native range.

Relation of clipping and drought to heat resistance.—The curves of figures 1 to 4 and the analyses of table I also contain the data on the effect of clipping and watering treatments on heat resistance. Clipping reduces the photosynthetic area, and watering favors the use of carbohydrates in growth. The unclipped and sparingly-watered plants would have been expected, therefore, to accumulate a greater percentage of carbohydrates. Data to be presented later show that such an accumulation occurred in both the greenhouse experiments and in unclipped or ungrazed grasses in the field during drought. Carbohydrate accumulations should stimulate the differentiation (hardening) of protoplasm (**14, 15, 22, 44**) and thus its resistance to heat. The data presented indicate that hardening was a major factor in heat resistance. In all instances hardened plants were more resistant to heat than unhardened plants. The least difference was with slender wheatgrass. The probable reason for the lack of hardening in this species was its rather poor condition toward the close of the drought treatment; apparently the greenhouse temperature was too high. This effect tended to complicate the analyses of variance of the data. Nevertheless moisture condition accounted for a greater percentage of the total mean square than any other single factor (table I). Also, all interactions in which moisture was represented had a fairly large mean square.

Drought-hardened plants were more sturdy and tough. The rhizomes and crowns had considerably more outward protective cover in the form of scales. Microscopic examination revealed more lignification in hardened

plants, as would be expected (**22**). The protoplasm may be assumed to have possessed the hardened qualities common to drought or frost-hardened plants as found by several workers (**20, 21, 22, 38, 39**), which enabled them to resist heat to a greater extent than unhardened plants.

In the analyses of variance of the clipping and drought-treatment data, the effects of clipping proved highly significant by the *F* test (**9**). However, the magnitude of the mean square for clipping was far less than for species, moisture, or heat treatments. Two factors reacted to complicate the data in this analysis: (a) Bermuda and buffalo grass stool out near the ground when clipped, thereby retaining a considerable volume of foliage for manufacturing food supplies necessary in the hardening process; (b) the unclipped plants had to be clipped to a 4-inch height during the heat treatment. This severe clipping of these tall plants deprived them of the food-manufacturing ability necessary for rapid recovery. The injury was especially great for the two wheatgrasses which have sparse basal leaves. Considerably greater difference would probably have been shown in the heat resistance of clipped and unclipped plants had it not been for these effects.

RELATION OF GRAZING TO HEAT RESISTANCE.—The results of heat treatments of Kentucky bluegrass rhizomes collected from a heavily grazed pasture and from an adjacent protected area showed a decided difference in their ability to resist heat. Clipping supposedly has the same effect as grazing and is used to simulate grazing. No trampling is done in clipping, however, and the results of a two-month clipping do not show the accumulative effects of continued heavy grazing. Other factors, such as compacting of the soil, erosion, less water infiltration, less water-holding capacity, and the competition of ungrazed plants also act to the disadvantage of heavily grazed plants as compared to artificially clipped plants. Still another important factor working to the detriment of heavily grazed plants is the limited root and rhizome development of such plants (**3, 17, 41, 45**). Although clipping has similar effects on root and rhizome development, the result in short time experiments might be less severe than on heavily grazed range.

CARBOHYDRATE RESERVES

EFFECT OF CLIPPING AND DROUGHT TREATMENTS ON RESERVES.—Table II contains the averages of the carbohydrate analyses of Bermuda and buffalo grasses grown in the greenhouse under four different conditions (figs. 3 and 4 and table I). Sufficient material was not available for carbohydrate analyses of the other grasses used in the heat treatment experiment. Although the data are limited they give a good indication of the effect of clipping and water supply on the food reserves of the grasses tested.

Unclipped, drought-treated plants of Bermuda grass showed a greater accumulation of carbohydrate food reserves than plants of any of the other treatments. The difference between the two drought treatments was chiefly in the amounts of colloidal carbohydrates in the form of glucosans and reducing sugars. There was a steady decline in reducing sugars from the

unclipped, dry treatment down to the clipped and watered. Differences were not so noticeable in sucrose content. There were distinct differences in the quantity of colloidal carbohydrates stored in the four treatment lots, but the most significant difference lay in the spread between the reserves of drought-treated and watered plants. Differences were found between clipped and unclipped watered plants in all of the carbohydrate fractions. Most of these differences were statistically significant in spite of the variability of the material.

With buffalo grass the food reserve differences were chiefly between dry and watered plants although consistent differences for all carbohydrate fractions occurred between clipped and unclipped, watered plants as in Bermuda grass. The chief difference indicated for clipped and unclipped, dry

TABLE II

AVERAGES OF CARBOHYDRATE ANALYSES OF ROOT AND RHIZOME SAMPLES OF BERMUDA GRASS AND BUFFALO GRASS*

SPECIES AND TREATMENT	NON-COLLOIDAL CARBOHYDRATES			COLLOIDAL CARBOHYDRATES	TOTAL CARBOHYDRATES
	REDUCING SUGARS	SUCROSE	TOTAL NON-COLLOIDAL	GLUCOSANS	
Bermuda grass					
Dry and unclipped	245 ± 16	3868 ± 32	4114 ± 15	2737 ± 368	6841 ± 384
Dry and clipped	154 ± 4	3975 ± 86	4129 ± 82	1553 ± 368	5682 ± 287
Watered and unclipped	122 ± 22	3199 ± 90	3321 ± 68	326 ± 90	3648 ± 157
Watered and clipped	68 ± 12	2502 ± 166	2570 ± 154	224 ± 60	2794 ± 213
Buffalo grass					
Dry and unclipped	405 ± 13	4779 ± 535	5185 ± 548	650 ± 123	5884 ± 721
Dry and clipped	404 ± 50	4977 ± 815	5381 ± 865	416 ± 56	5797 ± 810
Watered and unclipped	254 ± 26	3393 ± 191	3647 ± 217	151 ± 75	3799 ± 291
Watered and clipped	195 ± 37	2386 ± 15	2581 ± 22	128 ± 61	2709 ± 39

* Milligrams per 100 grams green weight of tissue and standard error of the averages.

plants lay in the glucosan content which decreased consistently from the unclipped, dry plants down to the clipped, watered treatment. Buffalo grass showed considerably less accumulations of colloidal carbohydrates than did Bermuda grass. Negative results were obtained in fructosan tests for both grasses. Temperatures under which the plants were grown in the greenhouse were kept high for purposes other than this investigation. Respiration was therefore high and the accumulations of food reserves were probably less than would have been obtained with lower temperatures.

EFFECT OF GRAZING AND DROUGHT ON FOOD RESERVES OF RANGE GRASSES IN OREGON.—The data of tables III and IV show the effect of heavy and moderate grazing and drought on two important range grasses in Oregon. Samples collected before drought were from green but fully mature plants. Those collected after drought were from plants whose leaves had been dried by drought for several weeks.

The outstanding fact revealed is that moderately grazed, bluebunch wheatgrasses stored up large quantities of colloidal carbohydrates as it

TABLE III

AVERAGES OF CARBOHYDRATE ANALYSES OF ROOT SAMPLES OF BLUEBUNCH WHEATGRASS ON HEAVILY GRAZED AND MODERATELY GRAZED RANGE, BEFORE AND AFTER DROUGHT*

CONDITION	NON-COLLOIDAL CARBOHYDRATES			COLLOIDAL CARBOHYDRATES			TOTAL COLLOIDAL AND NON-COLLOIDAL
	REDUCING SUGARS	SUCROSE	TOTAL NON-COLLOIDAL	LEVULOSANS	GLUCOSANS	TOTAL COLLOIDAL	
Moderately grazed before drought	374 ± 26	916 ± 76	1290 ± 76	1232 ± 308	1138 ± 226	2371 ± 641	3661 ± 652
Moderately grazed after drought	570 ± 62	1156 ± 54	1726 ± 71	4356 ± 52	1862 ± 342	6219 ± 382	7945 ± 387
Heavily grazed before drought	400 ± 10	710 ± 93	1110 ± 30	178 ± 48	700 ± 117	878 ± 72	1988 ± 92
Heavily grazed after drought	430 ± 23	1351 ± 81	1781 ± 57	448 ± 48	921 ± 160	1370 ± 158	3151 ± 165
T VALUES—11 DEGREES OF FREEDOM							
Moderately grazed before drought compared to after drought	+2.92†	+2.59†	+ 4.18‡	+8.66‡	+1.77	+5.15‡	+5.64‡
Heavily grazed before drought compared to after drought	+1.20	+5.21‡	+10.42‡	+3.90‡	+1.11	+2.81†	+6.69‡
Moderately grazed after drought compared to heavily grazed after drought	−2.11	+1.97	+ 0.60	−55.1‡	−2.47†	−11.7‡	−11.4†

* Milligrams per 100 gm. green weight of roots and standard errors of the averages.
† Significant at the 5 per cent. level (T = 2.20).
‡ Significant at the 1 per cent. level (T = 3.11).

entered the drought period (table III). Nearly ten times as much levulosan was found in the roots of moderately grazed plants after drought as in heavily grazed plants. Glucosan content was approximately twice as great in moderately grazed plants, and total colloidal carbohydrates were over four and one-half times as great. No significant differences in non-colloidal carbohydrates were found between heavily grazed and moderately grazed plants after drought. Sugars accumulated in both treatments; these increases, however, were of small magnitude compared to increases in levulosans. No significant increase of glucosans was made in either moderately or heavily grazed plants during drought, but this fraction was significantly low in the closely grazed plants.

Bluebunch fescue (*Festuca idahoensis* Elmer.), on the same range as bluebunch wheatgrass, showed smaller accumulations of food reserves during drought (table IV). Levulosans increased significantly in moderately grazed plants and showed a slight decrease in heavily grazed plants. Increase in sucrose during drought was highly significant in moderately grazed plants, while a significant decrease was shown in heavily grazed plants. There were no important changes in reducing sugars. Glucosans made significant increases in heavily grazed plants only.

When food reserves of the moderately and heavily grazed plants were compared at the close of the drought period, all carbohydrate fractions were much higher in the moderately grazed plants. As adjudged by the T-test the differences were all significant at the 1 per cent. level. In moderately grazed plants reducing sugars were approximately twice as great, sucrose was about one and one-half times as great, glucosans nearly one and one-third times as great, and levulosans about 17 times the amount contained in heavily grazed plants. This great difference in levulosan content suggests that it may have a special significance in drought resistance of this plant.

Effect of clipping and drought on Virgin Range grasses in Utah.—The data of tables V and VI show the effect of one season's clipping and drought on the food reserves of bluestem and bluebunch wheatgrass growing on a virgin sagebrush-wheatgrass range of Utah. All plots in this investigation were in the same condition at the beginning of the experiment. The plots were side by side on the same exposure, elevation and soil type. Conditions on all plots were as near alike as could be obtained under field conditions, and all sampling was randomized. The data present a clear record of the effects of clipping and drought during one season. Figure 5A shows a part of the area used; figure 5B shows adjacent over-grazed range.

Differences in food reserves brought about by drought alone were highly significant increases of large magnitudes in all carbohydrate fractions except reducing sugars which showed a highly significant decrease. Apparently the reducing sugars not used in respiration were converted into more complex carbohydrates during drought. The larger food reserve accumulations were in the form of colloidal carbohydrates—both levulosans and glucosans. Indications are that sucrose also was stored as reserve food. Food reserve

TABLE IV

AVERAGES OF CARBOHYDRATE ANALYSES OF ROOT SAMPLES OF BLUEBUNCH FESCUE UNDER HEAVY AND MODERATE GRAZING, BEFORE AND AFTER DROUGHT*

CONDITION	NON-COLLOIDAL CARBOHYDRATES			COLLOIDAL CARBOHYDRATES			TOTAL COLLOIDAL AND NON-COLLOIDAL
	REDUCING SUGARS	SUCROSE	TOTAL NON-COLLOIDAL	LEVULOSANS	GLUCOSANS	TOTAL COLLOIDAL	
Moderately grazed before drought	630 ± 90	592 ± 61	1222 ± 91	128 ± 28	1325 ± 71	1452 ± 49	2674 ± 134
Moderately grazed after drought	472 ± 16	1146 ± 61	1618 ± 49	275 ± 44	1451 ± 10	1726 ± 41	3344 ± 81
Heavily grazed before drought	213 ± 22	852 ± 18	1065 ± 24	43 ± 14	939 ± 19	982 ± 27	2047 ± 13
Heavily grazed after drought	227 ± 24	761 ± 30	988 ± 44	16 ± 10	1130 ± 48	1146 ± 47	2134 ± 66
T VALUES—11 DEGREES OF FREEDOM							
Moderately grazed before drought compared to after drought	−1.73	+6.42‡	+3.83‡	+2.82†	+1.75	+4.28‡	+4.27‡
Heavily grazed before drought compared to after drought	+0.42	−2.56†	−1.53	−1.57	+3.70‡	+3.02†	+1.29
Moderately grazed after drought compared to heavily grazed after drought	−8.50‡	−5.95‡	−9.57‡	−5.75‡	−6.55‡	−9.33‡	−11.6‡

* Milligrams per 100 gm. green weight of roots and crowns and standard errors of the averages.
† Significant at the 5 per cent. level (T = 2.20).
‡ Significant at the 1 per cent. level (T = 3.11).

TABLE V

AVERAGES OF CARBOHYDRATE ANALYSES OF ROOT AND RHIZOME SAMPLES OF BLUESTEM FROM VIRGIN RANGE BEFORE DROUGHT AND FROM CLIPPED AND UNCLIPPED PLOTS AFTER DROUGHT*

CONDITION	NON-COLLOIDAL CARBOHYDRATE			COLLOIDAL CARBOHYDRATE			TOTAL COLLOIDAL AND NON-COLLOIDAL
	REDUCING SUGARS	SUCROSE	TOTAL NON-COLLOIDAL	LEVULOSANS	GLUCOSANS	TOTAL COLLOIDAL	
Virgin before drought	351 ± 6	468 ± 25	819 ± 21	1066 ± 78	1227 ± 33	2282 ± 51	3111 ± 65
Virgin after drought	239 ± 7	1999 ± 44	2243 ± 32	3418 ± 75	3090 ± 151	6508 ± 190	8752 ± 236
Clipped plots after drought	242 ± 4	831 ± 13	1073 ± 12	216 ± 31	1278 ± 39	1494 ± 14	2568 ± 41
T VALUES—5 DEGREES OF FREEDOM							
Virgin before drought compared to virgin after drought	−12.2†	+30.0†	+32.6†	+21.8†	+12.1†	+21.9†	+23.0†
Virgin before drought compared to clipped after drought	−15.1†	+12.4†	+10.5†	−10.1†	+ 1.0	−16.1†	− 7.1†

* Milligrams per 100 gm. green weight of roots and rhizomes with standard errors.
† Significant at the 1 per cent. level (T = 4.032).

TABLE VI

AVERAGES OF CARBOHYDRATE ANALYSES OF ROOT SAMPLES OF BLUEBUNCH WHEATGRASS FROM VIRGIN RANGE BEFORE DROUGHT AND FROM CLIPPED AND UNCLIPPED PLOTS AFTER DROUGHT*

CONDITION	NON-COLLOIDAL CARBOHYDRATE			COLLOIDAL CARBOHYDRATE			TOTAL COLLOIDAL AND NON-COLLOIDAL
	REDUCING SUGARS	SUCROSE	TOTAL NON-COLLOIDAL	LEVULOSANS	GLUCOSANS	TOTAL COLLOIDAL	
Virgin before drought	234 ± 7	529 ± 31	763 ± 25	1334 ± 116	1437 ± 29	2771 ± 108	3534 ± 143
Virgin after drought	191 ± 5	1272 ± 18	1463 ± 12	2900 ± 157	3066 ± 116	5950 ± 125	7414 ± 131
Clipped plots after drought	241 ± 2	810 ± 4	1050 ± 2	1043 ± 47	1652 ± 97	2695 ± 149	3746 ± 154
T VALUES—5 DEGREES OF FREEDOM							
Virgin before drought compared to virgin after drought	−5.07†	+20.7†	+25.3†	+8.04†	+13.6†	+19.5†	+19.9†
Virgin before drought compared to clipped after drought	+0.96	+ 9.0†	+11.4†	−2.32	+ 2.11	+ 0.41	+ 0.95

* Milligrams per 100 gm. green weight of roots with standard errors.
† Significant at the 1 per cent. level (T = 4.032).

accumulation and the formation of colloidal carbohydrates during drought was the same for bluestem and bluebunch wheatgrass. The data from these species agree in general also with that presented earlier for the other grasses included in this study.

The striking result of clipping combined with drought was the prevention of any build-up of either levulosans or glucosans. In the clipped bluestem plants levulosans not only failed to increase during drought but dropped to 20 per cent. of their original value. The only significant increase in clipped

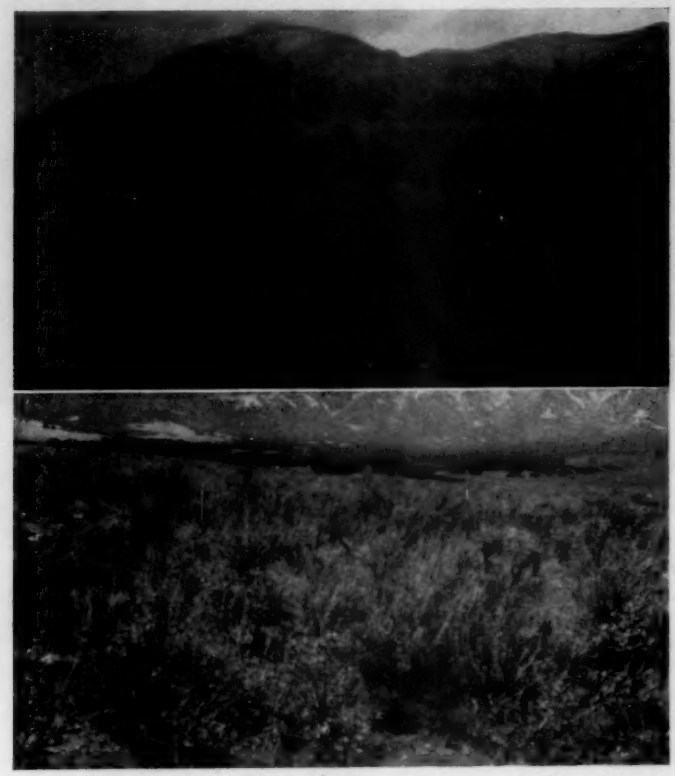


FIG. 5. Above—virgin sagebrush-wheatgrass range near Ogden, Utah; abundant wheatgrass and scattered sagebrush. Below—adjoining, over-grazed area; mostly sagebrush and downy chess.

plants of either species during drought was in the amount of sucrose present. Clipped bluestem after drought showed less than one-third as much total carbohydrate as unclipped plants. With clipped bluebunch wheatgrass the total carbohydrates were less than half those of the unclipped plants. Colloidal carbohydrates totaled over four times as much in unclipped bluestem as in clipped plants and the difference was more than twice in bluebunch wheatgrass. Colloidal carbohydrates are often reported as starch in the analyses of grass samples. Close microscopic examinations of all species analyzed were made under polarized light. Starch grains were observed in

several of the grasses, but they were extremely rare. The formation of starch seems to be possible in grasses, but in the species studied it was not an important form of carbohydrate storage in the vegetative organs. Iodine tests were negative for starch but indicated the common occurrence of dextrins in the grasses studied. These findings agree with those of VASSILIEV and VASSILIEV (**44**) who found starch present in the vegetative organs of wheat but in very small amounts.

EFFECT OF VEGETATIVE COVER ON SOIL TEMPERATURES

The results of the heat resistance studies prompted a third series of experiments. Since over-grazing is closely associated with drought injury,

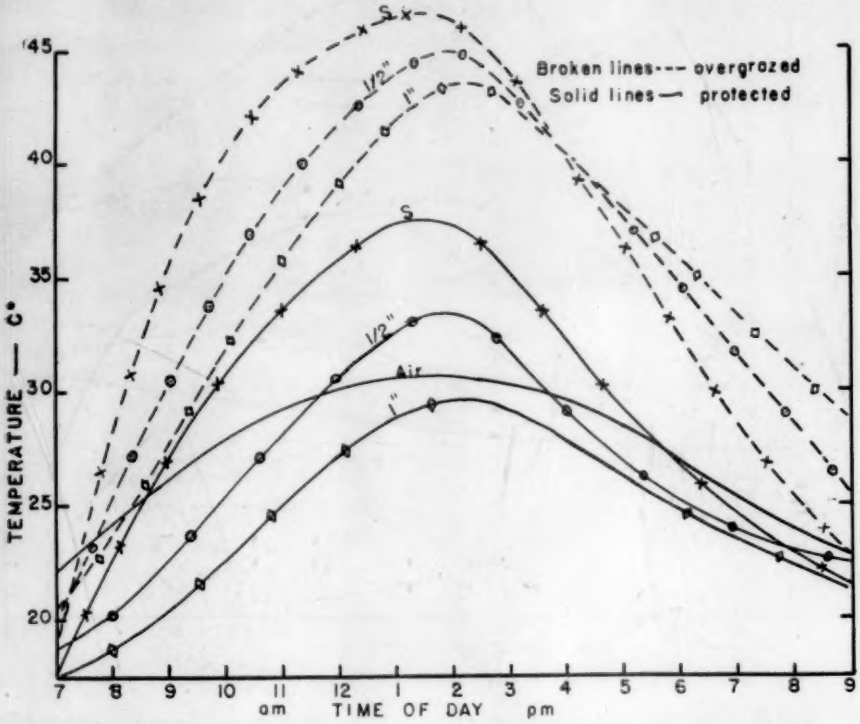


FIG. 6. Soil temperatures at 0- to 1-inch depths on over-grazed and protected, semi-desert grassland.

and clipped plants were less resistant to heat, it is possible that the reduced soil protection on an over-grazed range may contribute directly to grass injury by allowing higher soil temperatures. These effects would be in addition to the reduced resistance of closely grazed plants and would compound the injurious effects of overgrazing. Preliminary measurements on a mountain, bluegrass range showed soil temperatures in closely grazed areas several degrees higher than where the grass covering was less disturbed. Measurements on clipped and unclipped virgin range in Utah showed soil temperature differences of nearly 10° C. Measurements under more critical conditions were obtained on a semi-desert range in New Mexico.

SOIL TEMPERATURES ON OVER-GRAZED AND PROTECTED SEMI-DESERT GRASSLAND OF NEW MEXICO.—The semi-desert grass plots were selected to obtain typical over-grazed range for comparison with the same type range in good condition. The protected plot along a railroad right-of-way had a good stand of alkali sacaton (*Sporobolis airoides*), with an average vegetative soil cover of about 65 per cent. The over-grazed plot just through the fence supported only about one-tenth as much vegetative cover, and part of this was undesirable weeds and half-shrubs. The over-grazed plots differed further in having from one to two inches of top soil lost by recent erosion, as indicated by the heights of grass pedestals. Soil on the protected area had more pro-

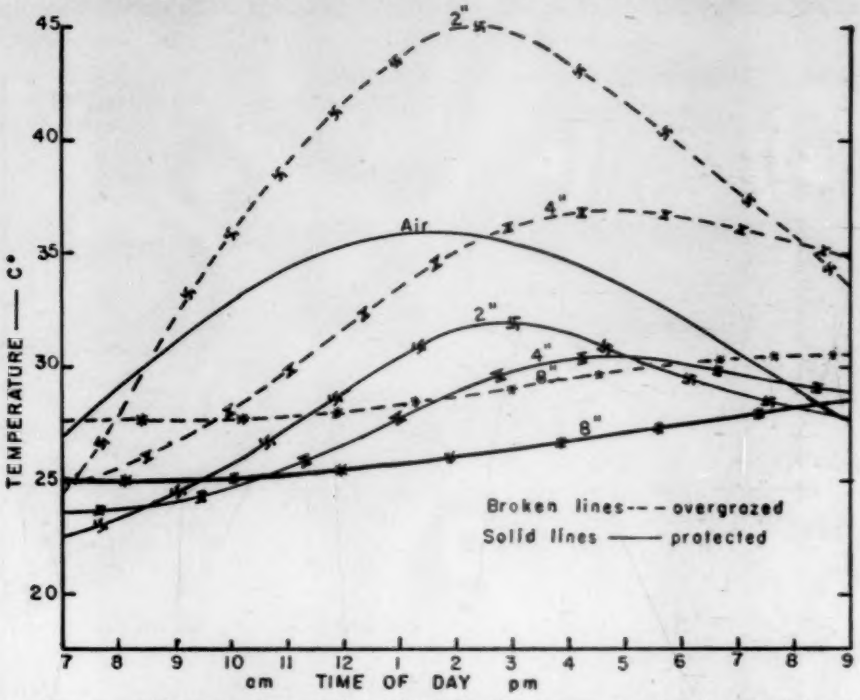


FIG. 7. Soil temperatures at 2- to 8-inch depths on over-grazed and protected, semi-desert grassland.

tective cover in the way of undecayed dead vegetative matter as well as greater organic material in the top soil.

Figures 6 and 7 show three-day average soil temperatures on over-grazed and protected, semi-desert grass plots and present a graphic comparison of the soil temperatures at various depths and the air temperatures at different times of day. Figure 6 compares the soil temperatures at surface, ½-inch, and 1-inch depths. In all cases temperatures on the over-grazed areas were considerably higher. Maximum soil temperatures for the day on the over-grazed surface were about 9 degrees higher, at the ½-inch depth over 11 degrees higher, and nearly 14 degrees higher at the 1-inch depth than temperatures at similar depths on the protected area. At the 2-inch depth

(fig. 7) soil temperatures averaged about 13 degrees higher on over-grazed range. At the 4-inch depth a difference of about 6½ degrees at 4 or 5 P.M. was found. At the 8-inch depth, temperatures between over-grazed and protected areas differed about 3 degrees throughout the day. Not only were soil temperatures higher on the over-grazed area, but the high temperatures extended over a longer period of time because they rose much more rapidly in the morning and declined less rapidly in the evening. A wide difference in soil temperatures therefore existed from shortly after sunrise until after sunset.

There was a distinct lag in the time at which different soil depths reached maximum temperatures during the day. Surface temperatures reached their maximum between 1 and 2 P.M. at the time of maximum air temperatures. Maximum temperatures for other depths were as follows: 2 P.M. for the ½-inch depth; about 2:30 P.M. for the 1-inch depth; between 2 and 3 P.M.

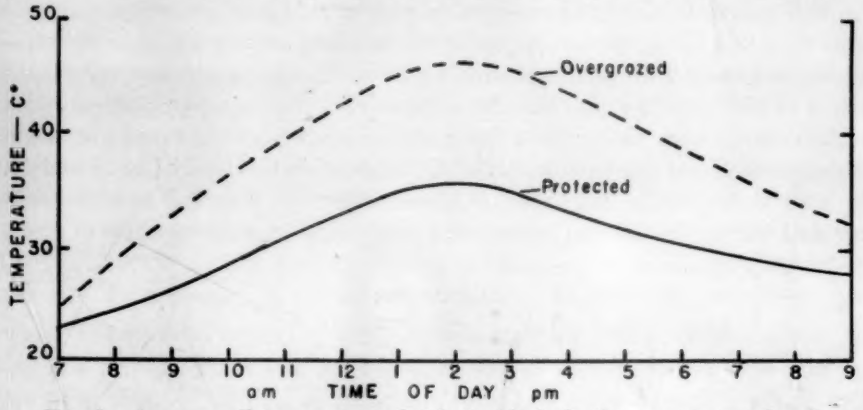


FIG. 8. Average soil temperatures for 0- to 4-inch depths on over-grazed and protected, semi-desert glassland.

for the 2-inch depth; about 5 P.M. for the 4-inch depth; and not until 8 or 9 P.M. for the 8-inch depth.

Soil temperatures on the over-grazed area from the surface to the 2-inch depth were much higher than air temperatures, reaching what are commonly considered lethal or near-lethal temperatures for plants, especially at the surface, ½-inch, and 1-inch depths where they reached a maximum of 51.5, 50.0, and 48.5° C., respectively. Even at the 4-inch depth, soil temperatures reached a higher maximum than air temperatures. Soil temperatures on the ungrazed area were below air temperatures except at the surface and ½-inch depths. Even these shallower depths did not reach dangerously high maxima. Figure 8 gives a comparison between average temperatures for the first four inches of soil on over-grazed and protected areas. A substantial difference is shown throughout the day and as much as 10° C. at midday.

Temperature and other drought conditions were not excessive during the period of study. Maximum air temperatures were slightly under 36° C. (96.8° F.), whereas during periods of severe drought temperatures of over

43.35° C. (110° F.) are not uncommon. SAVAGE (36) reported that the average maximum temperature for a sixty-day period at Woodward, Oklahoma, was 100.6° F. during the summer of 1934. At higher air temperatures the soil temperatures should be proportionately greater. Air temperatures of 100° F. and over could be expected to result in soil temperatures reaching a critically high stage to a 4-inch depth and maintaining it for a period of several hours each day.

On the semi-desert area studied, most of the grass roots occurred in the first six inches of soil, although a few roots reached a depth of several feet. Root-crowns and stolons are at or near the ground surface, and most grass rhizomes are in the first inch of soil. These vital plant parts, as well as the mass of feeder roots, are exposed to high soil temperatures in periods of hot weather and drought. This exposure is undoubtedly an important factor contributing to the high mortality of grasses during drought years.

Differences in soil temperatures on over-grazed and protected range as shown in this investigation are sufficient to affect greatly the rate of respiration and other vital processes within plants. Temperatures on over-grazed range reached levels which have been reported lethal to other plants. Such temperatures were higher than those which killed heavily grazed grasses in experimental heat treatments previously reported in this study (figs. 1 and 4). It is very reasonable, therefore, to assume that the high soil temperatures reached during drought on over-grazed range may be a direct cause of death of forage plants.

Discussion

The survival of different species on semi-desert ranges subjected to heavy grazing may be determined by the growth form and other natural adaptation of the plants to withstand close cropping, as well as by their inherent ability to resist heat and dehydration. Drought-resistant, tall or mid-grasses of the bunch type are often replaced by short grasses or sod-forming grasses which are better able to resist heavy grazing. On the Coconino Plateau of northern Arizona blue grama has replaced the climax bunch-grasses on heavily grazed mountain parks. The results of this study indicate that the greater drought resistance of such short grasses on heavily grazed, arid ranges may be due largely to their ability to maintain a considerable photosynthetic area, for manufacturing food reserves for protoplasm hardening in spite of close grazing.

Critically high temperatures generally accompany extended drought and injure forage plants by: (a) increasing respiration, and thus increasing food requirements; (b) increasing transpiration and evaporation which decreases the amount of water available; and (c) causing direct heat injury or death. Since respiration is increased by high temperatures during drought, a good supply of reserve food is necessary to support respiration as well as to enable roots, crowns, and rhizomes to become hardened to withstand heat and dehydration. MCCARTY (26) and MCCARTY and PRICE (27) have emphasized the necessity of limiting fall grazing to permit autumn

food reserve storage for winter hardiness and for vigorous spring growth. On semi-desert ranges, drought is much more destructive than frost. Food storage for drought hardening is therefore even more important than reserves for winter hardiness in these areas.

The results of this study emphasize the importance of moderate grazing before drought. Such practice permits accumulation of food reserves for drought hardening and the residual vegetation forms a protective cover which prevents excessive soil temperatures and reduces run-off and erosion. The result is less severe drought conditions together with hardened plants which are better able to resist heat and dehydration and are capable of rapid recovery with the return of good growing conditions. Over-grazing on arid ranges initiates a vicious cycle. The more a range is over-grazed the more severe drought conditions become and the less are forage plants able to resist heat and dehydration. Increased competition of unpalatable non-forage plants adds further complications to drought survival on over-grazed range. Still another serious handicap of over-grazed plants is the limiting of root growth by close cropping (**45**). LANTOW and FLORY (**17**) estimated that blue grama grass roots in full vigor had a root penetration of $2\frac{1}{2}$ to $3\frac{3}{4}$ feet. Those of low vigor reached a depth of 1 to $2\frac{1}{2}$ feet with an estimated volume of $\frac{1}{3}$ that of full vigor roots. Plants of depleted vigor had a root depth of $\frac{1}{2}$ to $\frac{3}{4}$ feet with only about $\frac{1}{10}$ of the root volume of full vigor plants. Not only is root volume and penetration restricted, but the amount of water in the soil is limited because of increased run-off and decreased water holding capacity of the soil resulting from erosion. The above factors working together inevitably result in serious depletion of both forage cover and site conditions.

Regulating grazing to meet plant requirements during drought years is one of the most difficult problems in sustained yield range management. This fact is reflected in the depleted condition of a large proportion of the semi-desert ranges of the west. Growing conditions fluctuate radically from year to year, and drought alone is often severe enough to cause considerable depletion in vegetative cover. Common practice on many private and unregulated ranges during drought is to hold over as many livestock as will live through the dry period without starvation. Such practice works directly contrary to forage plant requirements. The more severe the drought the heavier the chief forage plants are grazed, and the heavier they are grazed the less drought they are capable of withstanding.

Regulating numbers of livestock to meet plant growth requirements is especially difficult on year-long ranges where livestock depend entirely on the range for forage. To meet this problem the U. S. Forest Service recommends stocking and breeding herd on the basis of 10 to 30 per cent. below the carrying capacity of the average year, depending upon the frequency and severity of drought conditions. They also recommend some fluctuating of livestock numbers to meet current changes in forage supply. Under this management the good years are supposed to offset the bad effect of drought

years. LANTOW and FLORY (17) of the Soil Conservation Service advise going all the way in fluctuating livestock numbers to meet the current forage supply. Theoretically this system is adapted to meet plant requirements during drought. The practicability of the system is questionable, however, on ranges where supplemental feed is not available at a reasonable cost. On some ranges of the Southwest practically no growth is made during extreme drought years. To meet survival requirements of key forage plants no grazing should be permitted during such years and no breeding stock could be carried over. The system of fluctuating livestock to meet current forage supplies is very desirable from the standpoint of plant requirements and is good practice as far as practical application permits.

The results of this study confirm, from a drought-resistance standpoint, the soundness of some of the main principles of range management as developed by the U. S. Forest Service; namely, the practice of moderate grazing, proper season of use, and deferred and rotation grazing. Deferred and rotation grazing as commonly practiced on national forest ranges to insure seed production and natural reproduction is also beneficial in drought resistance. When grazing is deferred until after seed maturity the plants are in good condition to resist drought. Since the deferring of grazing is rotated from year to year on different units, the entire range benefits from a drought-resistance standpoint. CRADDOCK and FORSLING (8), working on a spring-fall sheep range of southern Idaho, found that the deterioration in the vegetative cover of important forage plants varied directly with the intensity of early spring grazing. This area is subjected to frequent droughts, and in this case too-early grazing was apparently an important factor contributing to drought destruction of the important forage plants.

Application of the results of the carbohydrate studies before and after drought, and of the investigation on the influence of food reserves on heat resistance, to range management calls for special utilization standards for important forage plants on arid ranges to be applied at the beginning of the usual dry period. Leaf area is the key to drought hardiness of forage plants. It is realized that regulating proper use at the beginning of the dry season is not without complicated problems, but drought destruction of major forage plants will be alleviated to the extent to which this practice is followed.

Summary and conclusions

1. A study was made of drought factors affecting range grasses as follows: (a) Heat-resistance tests were made with five grasses grown under four different conditions to determine the effect of food reserves on heat resistance. Supplementary tests were also made with heavily grazed, clipped, and protected plants grown in the field. Plants, protected by glass tubes, were immersed in a constant-temperature bath and exposed for ½ to 16 hours to a temperature of 48° C.; (b) Carbohydrate analyses were made of root and rhizome samples of range grasses to determine the effect of heavy grazing and clipping upon food reserves before and after drought,

and to investigate the rôle of the various carbohydrate fractions in drought resistance; (c) Soil temperatures were taken on over-grazed and protected range and on clipped and unclipped virgin range to determine the effect of vegetative cover and over-grazing on soil temperatures.

2. There were highly significant differences in the ability of the grasses tested to resist heat. Buffalo grass and Bermuda grass were most resistant, bluestem was intermediate and slender wheat, smooth brome, and Kentucky bluegrass were low in resistance.

3. Accumulation of food reserves was essential to heat resistance. All species tested when low in food reserves and unhardened were very susceptible to exposures of 48° C. Heat resistance increased with an increase in food reserves.

4. Results of this study indicate that heat resistance is a measure of drought resistance. The ability of the species tested to resist heat corresponds closely with the aridity of their natural habitats. Further tests with additional species are necessary for more definite conclusions.

5. Hardening by drought, under conditions favoring accumulation of reserves, produced highly significant differences in the ability of grasses to resist heat. Drought-hardened plants were much higher in food reserves than unhardened plants and were more resistant to heat injury in all comparisons.

6. The detrimental effects of clipping on heat resistance proved highly significant when the data on all species were pooled. Bermuda grass, buffalo grasses, and bluegrass, however, were much more resistant to clipping than other species. By stooling out near the ground these grasses were able to maintain sufficient foliage, even under moderately heavy clipping, to provide reserve accumulations for drought hardening. Heavy grazing of bluegrass resulted in a decrease in heat resistance.

7. Samples taken before and after drought revealed that protected or moderately grazed grasses accumulated excess food reserves as they entered drought. Large accumulation of colloidal carbohydrates, especially levulosans, was associated with drought resistance. Over-grazed and heavily clipped plants did not accumulate food reserves during drought and were less resistant.

8. Bodies thought to be starch grains were observed in several grasses, but this form of carbohydrate was extremely limited in the species studied. Sucrose accumulations were found whenever large supplies of colloidal carbohydrates were present, but reducing sugars did not accumulate during drought.

9. Several of the chief principles of range management as developed by the U. S. Forest Service are substantiated by the results of this study from a drought-resistance standpoint.

10. Application of the results of this study calls for further adjustments in range management to provide proper utilization standards for important forage plants at the beginning of the usual dry season. Such practice would

provide for: (a) hardened plants capable of withstanding drought; (b) adequate food reserves for respiration and for vigorous recovery after drought; and (c) reduction in drought conditions by a protective vegetative cover which guards against high soil temperatures and water loss.

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A DIBASAL (MINIMUM SALT, MAXIMUM YIELD) SOLUTION FOR *ASPERGILLUS NIGER;* ACIDITY AND MAGNESIUM OPTIMUM

ROBERT A. STEINBERG

Introduction

Two major difficulties exist in the use of *Aspergillus niger* v. Tiegh for identification and study of the rôles of micronutrients, although these are certainly not unique with this fungus. The water, sucrose, and salts employed may be assumed never to be completely free from impurities of micronutrients. Moreover, increases in yield with apparently non-nutritive elements may be due to biological substitutions and other obscure phenomena and not to essentiality. Decision in some instances is difficult or impossible.

These points have been considered by the writer at various times in the past. It was noted that better provision of micronutrients were paralleled by decreased macronutrient needs (**2**). Addition of traces of organic compounds to the nutrient solution gave no increases in yield except when micronutrients were deficient in quantity for maximum yield (**3**). Deficiencies in potassium or magnesium led to similar increases in yield with sodium and beryllium (**5**), respectively. Similar results (unpublished) have also been obtained with other apparently non-nutritive elements (Ti, Cb, Pd, etc.) when micronutrients were insufficient for maximum growth. These studies and the present one have been a direct consequence of the effort to obtain sufficient information to serve as a basis for interpretation of such anomalous increases obtained with apparently nonessential elements. The increases were often the results of actual or induced deficiencies in known nutrient elements and not of nutritive functions of the elements under trial.

The phenomena under discussion have also led to a quantitative study of the actual minima of the nutrient elements necessary for maximum yield. Further data are herein presented and should be interpreted particularly in the light of PFEFFER's (**1**) conclusion with respect to the mineral nutrition of (higher) plants: "All the elements cannot . . . be simultaneously reduced to the lowest possible minima" in the nutrient solution if growth is to be maximum.

Briefly stated, the data herein presented comprise a quantitative comparison of the optima obtained for all the macronutrient elements in quantity-yield experiments with those actually needed in the dibasal nutrient solution. Included also are brief but pertinent data on the relation of these results to micronutrient requirements, and the relation of nutrient solution acidity (pH), chloride, and of quantity and source of inoculum to deficiencies, whether actual or induced.

Methods

Aspergillus niger v. Tiegh was grown at 35° C. for 4 days on 50 ml. of nutrient solution in 200-ml., quartz, Erlenmeyer flasks. The cultures were

weighed after filtration and drying at 103–5° C. Acidities were determined with a Beckman pH meter, provided with glass electrode. Precision in determining yields may be assumed to be ± 5 per cent., since 95 per cent. of duplicate cultures agree to this extent (**6**). Numerous repetitions of experiments with nutrient solutions differing slightly in quantities of components served as additional checks (consistency in results). Other features of technique will be found in a previous publication or the text.

Results

Minimum requirement for magnesium sulfate

During the course of the writer's studies of trace element requirements, certain discrepancies were disclosed from time to time. Most prominent of these perhaps was that magnesium sulphate was required in larger quantity in the nutrient solution than in determinations of the individual optima for magnesium and sulphur. The values concerned will be found in table III under "old dibasal" solution and "unit optima" dibasal.

Table I contains a summary of these discrepancies with magnesium sulphate as concerns yields. The maximum yield in the old dibasal solution with 250 mg. of $MgSO_4 \cdot 7H_2O$ per liter was 1174.0 mg. per 50 ml. of solution, and only 1075.1 mg. with 190 mg. of $MgSO_4 \cdot 7H_2O$. An increase in hydrogen ion concentration brought the latter yield to 1227.8 mg. The use of K_2HPO_4 in the old dibasal solution and the consequent high pH (7.0 ±) appeared related to the higher magnesium sulphate requirement.

This experiment was verified in the last 5 experiments of table I where similar results were obtained with HCl and also with equivalent quantities of chloride ion as NH_4Cl or KCl, but not NaCl. (Discussion of the salt effects as well as those on micronutrient deficiencies will be postponed for another section of this text.) These data leave little doubt that 10 mg. of magnesium and 25 mg. of sulphur per liter (the quantities found in unit optima studies) are actually sufficient for maximum yield regardless of hydrogen ion concentration.

The dibasal nutrient solution

A summary of data on the minimum salt requirements for maximum yield is given in table II. The quantities of salts in the old dibasal solution are shown in column 1, and underneath is the quantity of each element present in the solution. To the right is the percentage of each element absorbed from the old dibasal solution. If the quantities absorbed from this solution are taken as the basis for a minimum salt solution, the values tabulated under "absorption dibasal" are obtained. If the solution quantities are computed on the basis of individual determinations of the optima for each macronutrient, the values in the last column are obtained.

Attention is called to the fact that though the old dibasal solution required a minimum of 24.8 mg. Mg and 32.5 mg. sulphur per liter for maximum growth, the quantities actually used by the fungus were only 11.5 and 23.0

TABLE I

YIELDS OF *Aspergillus niger* (4 DAYS, 35° C.) IN THE OLD DIBASAL NUTRIENT SOLUTION AND THE EFFECTS OF MAGNESIUM CONTENT AND ACIDITY ON MICRONUTRIENT DEFICIENCIES

Element omitted	Old dibasal solution*									Solution with minimum Mg (9.9 mg./l.) and S (25.1 mg./l.)														
	24.7 mg. Mg/l. 32.5 mg. S/l. pH = 6.69			18.7 mg. Mg/l. 24.7 mg. S/l. pH = 7.15			18.7 mg. Mg/l. 24.7 mg. S/l. pH = 3.18			Control pH = 7.13			HCl (27.2 mg. Cl/l.) pH = 6.67			NH_4Cl (29.2 mg. Cl/l.) pH = 7.13			NaCl (29.2 mg. Cl/l.) pH = 7.13			KCl (29.2 mg. Cl/l.) pH = 7.13		
	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†	Yield	Relative yield	Sporulation†
	mg.	%		*mg.*	%		*mg.*	%		*mg.*	%		*mg.*	%		*mg.*	%		*mg.*	%		*mg.*	%	
None	1154.9	100.0	8	1053.0	100.0	8	1209.2	100.0	10	839.3	100.0	4	1070.5	100.0	9	1034.7	100.0	6	971.2	100.0	6	1101.4	100.0	9
Fe	128.5	11.1	2	129.7	12.3	2	399.7	33.1	2	104.7	12.5	2	196.2	18.3	2	93.9	9.1	2	73.9	7.6	1	96.3	8.7	1
Zn	32.4	2.8	2	32.2	3.0	4	137.5	11.4	8	216.4	25.8	10	109.5	10.2	8	116.2	11.2	8	95.3	9.8	6	139.5	12.7	4
Cu	1157.9	100.3	6, t	1053.0	99.5	2, br	1227.8	101.8	6, br	977.4	116.4	2	1043.0	97.4	4	923.4	89.2	2	978.1	100.7	2	926.2	84.1	1
Mn	479.2	41.5	0	395.2	37.4	0	397.3	32.9	0	397.9	47.4	0	445.8	41.6	0	428.6	41.4	0	313.0	32.2	0	307.3	27.9	0
Mo	707.2	61.2	8	616.4	58.3	4	808.1	66.8	2	655.3	78.1	4	692.4	64.7	4	650.5	62.9	4	715.9	73.7	4	633.1	57.5	2
Ga	1112.0	96.3	8	967.6	91.5	4	1211.2	100.2	10	820.3	97.7	4	939.3	87.7	4	1007.2	97.3	6	804.5	82.8	4	972.3	88.4	4
Max‡	1174.0	.....	....	1075.1	.....	.....	1227.8	.....	.....	977.4	.....	...	1126.7	...	..	1094.7	.....	..	978.1	.....	...	1116.9	.....	..

* Water, 1000; sucrose, 50; NH_4NO_3, 1.90; K_2HPO_4, 0.35; $MgSO_4 \cdot 7H_2O$, 0.25 gm.; $MgSO_4 \cdot 7H_2O$, 0.19 gm. = 18.7 mg. Mg and 24.7 mg. S.
† Sporulation is indicated on a scale of 0 = sterile to 10 = completely covered with spores. Spore color was black, brown (br), or tan (t).
‡ Maximum individual yield.

mg., respectively. These values for actual absorption under an unfavorable low acidity correspond very closely with the values obtained in unit determinations; i.e., 11.5 and 10.0; 23.0 and 25.0. The unfavorable influence of high pH was on absorption and not requirements of the fungus.

These conclusions were subjected to further test in the experiments of table III. The composition of the "absorption" dibasal is based on the tacit assumption of 100 per cent. absorption of each nutrient element. That of the "unit optima" dibasal is based on actual determinations of the requirement for each nutrient element. Yields are almost maximum and almost identical for both solutions.

TABLE II

COMPOSITION OF DIBASAL SOLUTION DETERMINED EXPERIMENTALLY AS COMPARED WITH THAT COMPUTED FROM ABSORPTION DATA OR INDIVIDUAL OPTIMUM TESTS

Solution ingredients	Old dibasal (pH = 7.0 ±)		"Absorption" dibasal* (pH = 4.30)	"Unit optima" dibasal† (pH = 4.26)
	Quantity	Percentage absorption		
	mg./l.	%	*mg./l.*	*mg./l.*
NH_4NO_3	2500.0	.....	1781.0	1794.6
KNO_3	.....	.....	156.2	191.7
KH_2PO_4	.....	.....	263.1	263.7
K_2HPO_4	350.0	.....	.....	.....
$MgSO_4 \cdot 7H_2O$	250.0	.....	116.6	101.5
$(NH_4)_2SO_4$	.....	.....	32.1	48.4
N	665.0	0.980	651.7‡	665
K	157.2	0.865	136.0	150
P	62.3	0.961	59.9	60
Mg	24.8	0.464	11.5	10
S	32.5	0.709	23.0	25

* The quantities employed in this solution correspond to those absorbed from the "old dibasal" solution during growth of the fungus (6).

† The quantities used in this solution correspond to the optimum amounts of each element required for growth, as determined in individual quantity-yield tests.

‡ These values are products of quantity times absorption from the old dibasal solution.

Both solutions unexpectedly appeared slightly deficient in sulphur, and to the same degree. No further tests with sulphur were made, but trials were made with 19 trace elements, HCl, and increased inoculum with the "unit optima" solution. The yield was increased to 1209.8 mg. with 1.0 p.p.m. Na; 1162.7 mg. with 0.05 p.p.m. Ti; 1138.7 mg. with 0.05 p.p.m. Cb; and 1146.7 with 34,400 spores per flask. The spores were from cultures grown on Czapek agar.

The reasons for the increases caused by Na, Ti, and Cb require further study since sulphur appeared slightly deficient in quantity. Nevertheless, the results with sodium, titanium, and perhaps columbium surpass those with sulphur, in view of the quantities involved. Emphasis is placed on these data only for the purpose of pointing out that probably no real sulphur deficiency exists in these solutions. That is to say, the sulphur deficiency is an

TABLE III

COMPARISON OF MAXIMUM YIELDS AND OF THE EFFECTS OF MICRONUTRIENT DEFICIENCIES WITH TWO COMPUTED DIBASAL SOLUTIONS

Element omitted	"Absorption" dibasal						"Unit optima" dibasal					
	Czapek agar spores (430,000 per liter)			Synthetic solution spores (174,000 per liter)			Czapek agar spores (430,000 per liter)			Synthetic solution spores (174,000 per liter)		
	Yield	Relative yield	Sporulation*	Yield	Relative yield	Sporulation*	Yield	Relative yield	Sporulation*	Yield	Relative yield	Sporulation*
	mg.	%		*mg.*	%		*mg.*	%		*mg.*	%	
None	1084.2	100.0	6	1024.5	100.0	6	1084.8	100.0	7	980.1	100.0	6
Fe	60.9	5.6	1	47.4	4.6	1	67.9	6.2	1	64.0	6.5	1
Zn	57.5	5.3	4	63.4	6.2	4	47.8	4.4	4	45.3	4.6	2
Cu	1080.5	99.7	3	996.7	97.3	2	1044.1	96.3	2	1046.8	106.8	2
Mn	374.1	34.5	0	312.5	30.5	0	356.6	31.9	0	273.1	27.9	0
Mo	599.4	55.3	2	628.5	61.3	2	594.6	54.8	2	617.3	63.0	2
Ga	1092.2	100.7	6	971.0	94.8	4	1056.7	97.4	6	1029.0	105.0	4
Max.**	1108.6			1053.6			1114.2			1061.7		
C.U.†	44.3%			42.1%			44.6%			42.5%		
pH‡	4.30			4.30			4.26			4.26		
Deficiency§	+S = 1170.6 mg.			+S = 1101.1 mg.			+S = 1157.8 mg.			+S = 1121.3 mg.		

* See table 1, footnote.
** See table 1, footnote.
† Yield per 100 grams of sucrose.
‡ Initial acidity of nutrient.
§ Yields obtained by addition of a slight excess of $(NH_4)_2SO_4$ whereas NH_4NO_3 was ineffective.

induced one due to proportions still slightly imperfect, or that an unknown deficiency exists.

Each milligram of salt added sufficed for a maximum yield of 9.4 mg. with the "absorption" dibasal and 9.3 mg. with the "unit optima" dibasal diet. The data respecting micronutrient deficiencies and inoculum will be discussed in a later section.

Relation of acidity (pH) to growth

It was pointed out in an earlier section that acidity has a marked effect on the magnesium optimum for growth through its effect on absorption. None of the other macronutrients were so affected by acidity within the range covered. Addition of acid or of substances inducing acidity, either directly or indirectly, would, therefore, result in increased yields and perhaps be used as a basis for claiming essentiality or stimulation.

Although acids other than hydrochloric are also effective, it must be anticipated that differences will be found on further study. Aside from the possible varying effectiveness within the cell, the reduction processes therein must rapidly dispose of acidity due to nitric or sulphuric acid as compared to hydrochloric.

Examination of table I will disclose that ammonium chloride and potassium chloride were also effective in increasing yields, whereas sodium chloride was not. Their action is probably the result of absorption and utilization of nutrient ions (NH_4, K) by the fungus, thus causing residual acidity of the chloride ion to become effective. Increased yields, however, are due wholly to more effective absorption of magnesium and not to acidity in itself.

Some data have also been gathered on the effects of micronutrients on increases in yield with acidity under these conditions. The effects of increased concentrations of hydrochloric acid on yields varied greatly with even slight increases or decreases in proportions of micronutrients.

Responses to micronutrient deficiencies

The effects of micronutrient deficiencies were surprisingly uniform under all conditions encountered in these experiments. Omission of iron, zinc, manganese, and molybdenum resulted in sharply diminished growth under all conditions. Use of minimum quantities of magnesium and sulfur at pH 7 gave somewhat better results, particularly with copper and gallium in some cultures. Substitution of spectroscopically pure salts for the reagent chemicals would probably have resulted in still further improvement but was not attempted.

The spore factor

Inoculations with an excessively small quantity of spores give cultures of low yield quite susceptible to addition of trace elements. The increases in yield are erratic, however, and not consistently reproduceable. The quantity of spores required for maximum yield is affected by the medium on which they were produced. This is exemplified in table III containing final

experiments on size of spore inoculum. A total of 21,500 spores per flask from cultures grown on Czapek agar was found necessary to obtain maximum yields. Spores from cultures grown on the old dibasal solution for about 2 years (25 transfers) were even more effective since 8,700 spores per culture sufficed. Results of micronutrient tests were quite similar with spores from both sources.

The most effective spore concentration for maximum growth varies inversely with duration of growth. Organic substances, particularly glycollic acid and biotin, are also partially effective in minimizing the effects of low inocula.

Discussion

The major result obtained in this study consists in the proof that the dibasal nutrient solution for *Aspergillus niger*, and possibly for other organisms as well, contains quantities of all elements equal to or less than those determined as optimum in tests with the individual elements. That is to say, it is possible to obtain maximum yields when all elements are present in minimal quantities. This is the reason for terming the nutrient solution of minimum salt content for maximum yield a dibasal solution. It is basal both with respect to solution and organism (**4**).

This demonstration with Aspergillus throws doubt on the correctness of PFEFFER's interpretation that a nutrient solution of minimal quantities cannot produce maximum yield. Two outstanding sources of error existed in these older studies. The necessity for micronutrients was unknown, as was also the specific effect of chloride in maintaining acidity (optima for K, Ca, Mg were usually determined with the chloride).

Another important result consists in the possibility of computing the composition of the dibasal solution for other organisms from the values for absorption from an approximately dibasal solution. These values in turn could be determined approximately by estimations of the optima for growth in quantity-yield experiments with the individual elements.

In the foregoing discussion emphasis has been placed on the nutrient solution. It will be evident, however, that these data are in effect determinations of the minimal quantity of each nutrient element required for production per unit of yield. This necessarily follows since nutrients are at a minimum, yield is maximum, and absorption complete or practically so. Since each milligram of the macronutrient salt mixture supplied sufficed for 9.4 mg. of yield, each gram of fungus substance produced required a maximum of 106 mg. of macronutrient salts. If the value yield/salts be termed "efficiency of salt utilization," then its reciprocal is the "minimal salt requirement" per unit of yield.

The relation between magnesium absorption and acidity in contradistinction to the other macronutrients, while important in itself, is also important because of its implications. Acidification, for example, of a nutrient solution might lead to increased yields if magnesium absorption was previously insufficient for maximum yield, whereas it would be ineffective if the mag-

nesium content had been increased sufficiently to compensate for decreased absorption at high pH.

Variation in magnesium content of the nutrient solution is not the only means of altering apparent reactions of the organism to acidity. Very slight variations in micronutrient content were found to cause great alterations in the acidity-yield curves with Aspergillus at minimal magnesium content.

All increases in yield due to acidity do not therefore fall into the same category. Increases caused intrinsically by hydrogen ion doubtless occur, but not in the experience of the writer with Aspergillus (pH 3 to 8), which excretes much free organic acid. Increases resulting from induced deficiency of a nutrient ion, magnesium, or micronutrient have taken place. Induced nutrient deficiencies may be eliminated by an increase of the nutrient element in question (or a decrease in inhibiting ion in some instances?) whereupon the beneficial effect of acidity will also disappear.

The spores of this fungus are known to contain iron, copper, etc. It is therefore not surprising that the quantity used for inoculation of cultures should affect the degree of experimental micronutrient deficiency. Within limits better results are obtained with lesser quantities of spores. The use of still smaller inocula permitted by addition of glycollic acid or biotin (i.e., without reduction in maximum yields) did not, however, lead to improved deficiency results with micronutrients. Tests with other vitamins and other accessory growth substances were entirely negative.

Summary

Aspergillus niger v. Tiegh was grown at 35° C. for 4 days. Absorption of magnesium, but not of other macronutrients, was altered by variations in acidity. Values in acidity-yield curves depended on variations in magnesium and micronutrient content of the solution. The dibasal solution (minimum salt solution for maximum yield) was found to contain all the nutrient elements in minimal quantity, as determined in quantity-yield tests with the individual elements. Maximum yields, therefore, can be obtained when all essential elements are present in minimal quantity. The fungus requires not more than 106 mg. of macronutrient salts per gram of substance formed. Possible methods for computing the approximate composition of the dibasal nutrient solution for other organisms are indicated.

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EFFECTS OF DIFFERENT AMOUNTS OF POTASSIUM ON GROWTH AND ASH CONSTITUENTS OF *ANANAS COMOSUS* (L.) MERR.[1]

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(WITH EIGHT FIGURES)

Introduction

The effects of different amounts of potassium fertilizers on the growth and fruit yields of *Ananas comosus* have often been reported in the literature (**3, 16, 26**). Although such field studies have contributed valuable practical information, work on the physiological function of potassium in the metabolism of this plant has been limited.

Methods

The data reported in this paper were obtained from plants grown in nutrient solutions supplied with high or low amounts of potassium, namely 205 or 4 mg. per liter but with equal amounts of either nitrate- or ammonium-nitrogen.

Tops or crowns (vegetative organs produced at the apex of fruits) of uniform size from a single clone were placed in tap water for one month until they had produced about twenty roots of uniform thickness and length. Fourteen plants were then selected and transferred to each of the nutrient solutions described in table I. Each plant was grown in a 4-gallon porcelain crock containing 17 liters of solution which was constantly aerated and changed regularly at two-week intervals.

The plants were grown in these solutions for approximately one year, then all 14 were harvested, weighed, and sectioned according to previously reported techniques (**43**). The chemical methods employed for the analysis of the tissues were reported in previous publications (**39, 40, 41, 42**).

The statistical significance of the difference of the means of total plant weights between different cultures was calculated by FISHER's method (**6**, p. 14). However, the method of CONRAD (**5**) together with the Z table of LOVE (**20**) was employed for the calculation of the statistical significance of the differences of the means of the various plant constituents between different cultures.

In the presentation and discussion of results, various synoptic expressions have been introduced to replace longer appellations often repeated. For instance, the culture with 205 mg. of potassium per liter, or high potassium, will be designated by H-K; and that with 4 mg. of potassium per liter, or low potassium, by L-K. Also, the ammonium-nitrogen series will be referred to as A-n and nitrate-nitrogen as N-n.

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TABLE I

GRAMS OF NUTRIENT SALTS PER 100 LITERS OF SOLUTION AND CONCENTRATION OF ELEMENTS AS MICROGRAMS IN THE CULTURES DESIGNATED AS HIGH-K OR LOW-K AND SUPPLIED WITH EITHER NITRATE-IONS OR AMMONIUM-IONS AS SOURCES OF NITROGEN

Salts	Grams of salt per 100 liters of solution				Elements	Micrograms of elements per cc. of solution			
	Nitrate series		Ammonium series			Nitrate series		Ammonium series	
	High-K	Low-K	High-K	Low-K		High-K	Low-K	High-K	Low-K
	gm.	gm.	gm.	gm.		μ	μ	μ	μ
KH_2PO_4	6.81	1.36	6.81	1.36	K	205.0	4.0	205.0	4.0
K_2SO_4	41.39	.....	41.39	.....	Ca	40.0	40.0	40.0	40.0
$Ca(NO_3)_2 \cdot 4H_2O$	23.62	23.62	.....	.....	Mg	12.0	12.0	12.0	12.0
$MgSO_4 \cdot 7H_2O$	12.33	12.33	12.33	12.33	Fe	2.7	2.7	2.7	2.7
$NaH_2PO_4 \cdot H_2O$	.....	5.52	.....	5.52	Na	.....	101.2	.....	101.2
$Na_2SO_4 \cdot 10H_2O$	.....	64.44	.....	64.44	N	28.0	28.0	28.0	28.0
$CaCl_2$	.....	.....	11.10	11.10	S	93.6	81.6	125.6	113.6
$(NH_4)_2SO_4$	.....	.....	13.21	13.21	P	15.5	15.5	15.5	15.5
$FeSO_4 \cdot 7H_2O$	1.39	1.39	1.39	1.39	Cl	.....	.....	72.0	72.0

Results

Growth

Plant weights, indicating the growth attained in one year by the different cultures, are reported in table II and figure 1. The weights were greater in the H-K than L-K cultures, being 40 and 73 per cent. higher for the N-n and A-n series, respectively. The stems weighed more in the H-K than L-K cultures, being 94 and 185 per cent. greater for the N-n and A-n series. The

TABLE II

MEAN WEIGHT VALUES, WITH THE STATISTICAL SIGNIFICANCE OF THEIR DIFFERENCES, OF TOTAL PLANTS, STEMS, LEAVES AND ROOTS OF ONE-YEAR-OLD PLANTS (14 PLANTS PER CULTURE) GROWN IN NUTRIENT SOLUTIONS SUPPLIED WITH 205 OR 4 MG. OF POTASSIUM PER LITER, AND EITHER WITH NITRATE OR AMMONIUM SALTS AS SOURCES OF NITROGEN

Series	Organs	Mean weights		Difference	Significance of differences between means		
		High-K	Low-K		t	P =	In favor of
		gm.	gm.	%			
Nitrate-N	Total plants	3855	2745	40.4	4.00	< .01	High-K
Ammonium-N	" "	3895	2250	73.2	6.32	< .01	"
Nitrate-N	Stems	335	173	93.6	6.80	< .01	"
Ammonium-N	"	342	120	185.0	5.20	< .01	"
Nitrate-N	Leaves	3210	2280	40.8	10.80	< .01	"
Ammonium-N	"	3330	1820	83.0	15.90	< .01	"
Nitrate-N	Roots	314	288	9.0	1.00	> .30	None
Ammonium-N	"	197	310	57.4	5.50	< .01	Low-K

weights of the leaves, also, were greater in the H-K than L-K cultures, being 41 and 83 per cent. more for the N-n and A-n series. The weights of the roots had not been affected in the same way as those of the stems and leaves by the different potassium treatments. In the N-n series, the roots of the H-K cultures weighed about the same as those of the L-K cultures while in the A-n series these values for the L-K cultures were greater.

The differences of the means of the weights of the entire plants, stem and leaves were statistically significant in favor of the H-K cultures as indicated by the values of P in table II. The roots behaved differently; only the dif-

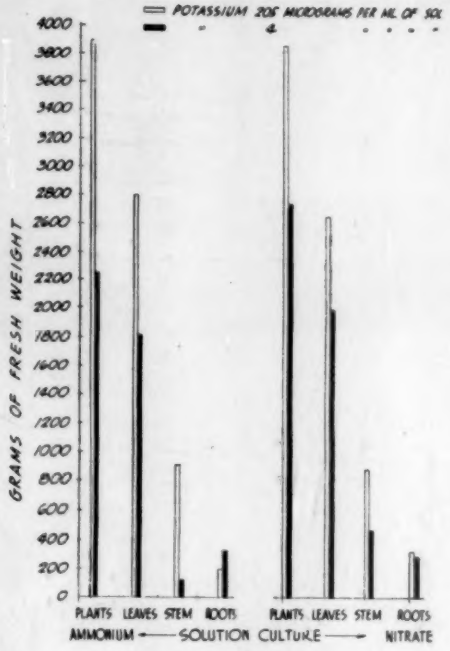

FIG. 1. Fresh weight yield of pineapple plants, age one year, in high and low potassium cultures.

ference of the means in the A-n series was significant in favor of the L-K cultures.

Leaf width, length, weight and area for the different cultures are reported in table III. These data show that the leaves of the H-K cultures were longer, wider, and consequently possessed a greater area and weight than those of the L-K cultures. Leaf widths were generally greater in the ammonium series and leaf lengths in the nitrate series, a condition also observed often in field grown plants. A product obtained by multiplying the area by the weight of the leaves of different groups, as reported previously (**43**), is used to compare the different groups with respect to mass of tissue and effective area involved. The values of this product in table III show that the leaves of the active (D) group constituted from 42 to 59 per

TABLE III

MEAN VALUES OF WIDTH, LENGTH, WEIGHT, AREA AND PRODUCT OF AREA × WEIGHT AND PERCENTAGE OF SAME OF DIFFERENT GROUPS OF LEAVES (B, C, D, E) OF *A. comosus* GROWN IN HIGH- OR LOW-POTASSIUM SOLUTION CULTURES AND SUPPLIED EITHER WITH NITRATE- OR AMMONIUM-NITROGEN

LEAF	NO_3								NH_4							
	HIGH-K				LOW-K				HIGH-K				LOW-K			
	B	C	D	E	B	C	D	E	B	C	D	E	B	C	D	E
Width (cm.)	5.14	7.40	7.32	4.15	4.64	3.29	6.46	3.06	6.98	7.43	7.43	5.35	6.02	5.70	5.96	3.79
Length (cm.)	51.6	90.0	96.5	45.2	46.5	73.0	90.5	35.2	67.1	83.8	88.8	55.8	54.1	67.6	74.7	40.0
Weight (gm.)	24.9	78.5	89.7	28.4	18.4	46.0	68.0	21.0	36.2	64.6	76.0	38.3	22.2	35.3	43.8	16.8
Area ($cm.^2$)	132	333	358	93	108	230	292	54	234	311	330	150	163	192	222	76
Product: (area×wt.)	3,287	26,140	32,113	2,641	1,987	10,580	19,856	1,134	8,471	20,091	25,080	5,745	3,619	6,778	9,724	1,277
Percentage*	5	41	50	4	6	32	59	3	14	34	42	10	16	32	46	6

* Percentage of the product of area × weight of all leaf groups.

cent. of the total and those of the mature (C) group from 32 to 41 per cent. In the N-n series the combined mature (C) and active (D) groups constituted 91 per cent. of the total leaf index and in the A-n series approximately 77 per cent. These comparisons show that differences in leaf length, width, area and weight between groups were greater in the N-n than A-n series.

Pathological symptoms of potassium deficiency were small brown discolorations in the chlorophyllose tissues and severe leaf tip necrosis which appeared in the L-K cultures when the plants were about seven months old. The latter symptom, developing first in the old leaves, extended in several cases about two-thirds the length of the entire leaf and gradually appeared in the leaves of the other groups in amounts directly proportional to their chronological age.

Water

The thickness of the water storage tissue varies in different sections of the same leaf of *A. comosus*, being always slightly greater in the low and middle chlorophyllose (nos. 3 and 4) than in other sections. Therefore, these sections may be employed more profitably than any others for the determination of the water content of plants subjected to different treatments.

The differences in the water content of the low and middle chlorophyllose sections (nos. 3 and 4) of the leaves between different treatments shown in table IV, all in favor of the H-K cultures, ranged from 0 to 4.7 per cent. in the N-n series and from 0.7 to 3.6 per cent. in the A-n series. They were greater mostly in the chlorophyllose (nos. 3, 4, 5) than in the basal or transitional (nos. 1, 2) sections of the leaves. It is possible that the differences in water content might have resulted from differences in the amounts of total organic solutes (sugars) which were greater in the L-K than H-K cultures. In the stem, the differences, mostly in favor of the L-K cultures, resulted from differences in the starch and protein content which was greater in the H-K than L-K cultures, as will be shown in subsequent publications.

Ash

The ash content, reported in table IV, was higher for the H-K than L-K cultures in both types of nitrogen series. The H-K cultures contained higher ash values in the N-n series than A-n series. Differences in the ash content of the L-K cultures between the N-n and A-n series were small in the leaves but relatively great in the stem.

Table V, reporting total ash values per plant, leaves, stem or roots, and figure 7, concentrations of ash in the tissues of the same calculated from the data in table V, show that the movement of ash constituents from roots to stem or from stem to leaves was greater in the H-K than L-K cultures. The ratios of stem to leaf ash and of stem to root ash, reported in figure 8 also indicating relative concentrations and suggesting trends of movement of ash, were greater in H-K than L-K cultures.

The low ash content of the leaves in the L-K cultures resulted, doubtless, from the low supplies of potassium in the nutrient solution which, after

TABLE IV

RELATIVE ELECTRICAL RESISTANCE (OHMS) OF THE SAP EXTRACT, ASH AND WATER PER GRAM OF FRESH TISSUE IN DIFFERENT SECTIONS OF *Ananas comosus* GROWN IN SOLUTION CULTURES CONTAINING EITHER 205 OR 4 MILLIGRAMS PER LITER AND EITHER NITRATE OR AMMONIUM SALTS AS NITROGEN SOURCES

PLANT SECTIONS	NITRATE SERIES						AMMONIUM SERIES					
	HIGH-K			LOW-K			HIGH-K			LOW-K		
	ASH	WATER	OHMS	ASH	WATER	OHMS	ASH	WATER	OHMS	ASH	WATER	OHMS
	mg.	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*		*mg.*	*mg.*	
Leaves:												
Old (B) 1+2 (base)	9.7	866	49	3.5	862	105	9.2	874	41	3.6	858	95
3	14.2	878	33	4.2	872	88	12.3	876	36	5.5	866	72
4	18.7	870	26	7.0	848	64	14.8	865	30	8.7	843	51
5 (tip)	27.0	850	22	6.7	840	58	17.1	836	28	9.5	830	50
Mature (C) 1 (base)	8.9	880	46	2.1	882	73	7.7	888	45	2.8	882	97
2	11.7	878	34	5.3	878	82	11.6	892	34	4.1	882	92
3	19.1	875	31	6.6	860	66	15.2	883	29	6.8	852	50
4	21.0	874	23	7.9	835	54	16.7	859	27	9.2	830	44
5 (tip)	25.7	845	19	9.6	821	52	17.7	827	25	9.5	822	41
Active (D) 1 (base)	6.5	904	56	3.9	903	79	4.5	913	62	3.4	901	85
2	10.6	882	43	4.3	883	81	8.8	887	44	3.8	881	86
3	14.9	882	33	6.8	862	68	12.2	880	35	6.3	856	54
4	20.8	856	24	7.4	843	58	16.6	860	27	8.0	831	44
5 (tip)	25.8	841	21	8.2	823	51	17.0	828	28	8.7	816	40
Young (E) 1 (base)	8.4	914	49	5.4	913	71	6.2	917	51	5.0	914	66
2	8.7	900	49	4.7	895	90	7.3	895	56	4.2	888	75
3	12.2	875	36	6.0	875	70	10.1	870	42	5.1	863	61
4 (tip)	17.1	849	27	7.2	837	56	11.8	831	36	5.9	835	48
Stem:												
Base	15.7	788	29	16.6	842	35	16.6	816	27	10.5	846	49
Middle	16.9	797	26	22.0	860	33	13.2	840	25	12.4	856	36
Apex	16.7	873	27	14.4	878	39	13.3	890	31	11.8	876	39
Roots:	6.8	851	81	4.5	834	100	6.8	851	55	3.8	863	102

TABLE V

TOTAL AMOUNTS OF ASH, POTASSIUM, CALCIUM, MAGNESIUM, PROSPHORUS AND IRON PER PLANT OR ORGAN OF *Ananas comosus* GROWN IN SOLUTION CULTURES WITH 205 OR 4 MICROGRAMS OF POTASSIUM AND WITH EQUAL AMOUNTS OF NITRATE- OR AMMONIUM-NITROGEN

ITEMS	NITRATE SERIES								AMMONIUM SERIES							
	HIGH-POTASSIUM				LOW-POTASSIUM				HIGH-POTASSIUM				LOW-POTASSIUM			
	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS	PLANT	LEAVES	STEM	ROOTS
Ash (mg.)	59,676	51,989	5,545	2,142	18,685	14,321	3,070	1,294	45,850	39,682	4,828	1,340	14,173	11,643	1,384	1,146
K (mg.)	43,553	39,179	3,602	772	5,972	5,557	277	138	39,445	34,630	4,362	453	7,129	6,728	289	112
Ca (mg.)	1,784	1,235	398	151	3,995	2,877	928	190	1,176	963	166	47	1,049	735	224	90
Mg (mg.)	696	478	114	104	1,421	1,325	73	23	884	676	151	57	1,192	922	140	130
P (mg.)	534	424	66	44	251	219	15	17	641	572	53	16	314	281	14	19
Fe (γ)	26,462	1,075	87	25,300	14,066	502	34	13,530	17,859	9,096	627	8,136	12,014	4,080	184	7,750
Wt. (gm.)*	3,855	3,210	335	314	2,745	2,280	173	288	3,895	3,330	342	197	2,250	1,820	120	310

* Weights of plant, etc.

absorption by the roots, moved to the leaves in amounts proportional to the concentration. The higher ash content in the stem than in the leaves of the L-K cultures resulted from the greater inflow of Ca and Mg in the L-K than H-K cultures, as shown below, which accumulated more in the stem than in the leaves and thus increased the total ash content of the former organ.

Electrical resistance

The relative electrical resistance (ohms) of the extracted sap was higher in the L-K than in the H-K cultures of both series, as shown in table IV. This condition resulted to a great extent from the lack of ample quantities of electrolytes and particularly of potassium in the L-K cultures. Comparisons of the values of ohms between the H-K cultures in the two series show that, with few exceptions, they were higher in the A-n series. This difference indicates that NH_4^+ and possibly the higher H-ion concentrations which formed in the ammonium series had partly inhibited the absorption of certain ions (Ca^{++} and Mg^{++} in figures 3 and 4) from these cultures. The values of ohms in the chlorophyllose sections of the leaves were greater in the N-n than A-n series of the L-K cultures, which is explained by the fact that the potassium content of the tissues was greater in the latter than former series. Gradient reversals in the ohm values between the basal (no. 1) and transitional (no. 2) sections of the mature (C), active (D), and young (E) leaves occurred more in the L-K than in the H-K cultures. They were doubtless associated with the concentrations of inflowing electrolytes and particularly potassium. With high concentrations of potassium as in the H-K cultures no gradient reversals developed.

Potassium

The tissues of the H-K cultures contained concentrations of potassium from 2.0 to 6.5 times as great as the L-K cultures, as reported in table V and figure 2. The data in table V, reporting the total amounts of potassium per plant, leaves, stem or roots, show that the H-K cultures contained 7.3 times more K in the N-n series and 5.6 times more in the A-n series than the L-K cultures. The concentration of potassium in the nutrient solutions was 50 times greater in the H-K than L-K cultures. However, the plants of the H-K cultures absorbed only approximately one-half the amount of potassium supplied in the nutrient solutions, whereas the plants of the L-K cultures had absorbed practically all traces of potassium at the end of two weeks when the nutrient solution was renewed. Therefore, the plants of the H-K cultures were supplied with amounts of potassium greatly in excess of the absorption capacity of the roots and possibly of the entire plant because analyses of the solution at the end of two weeks, the renewal interval, showed that less than one-half of the original amounts had been removed by the plants. However, similar analyses in the L-K cultures showed that these plants were supplied with amounts much lower than the capacity of the roots and entire plant because the entire K content of the nutrient solution had been removed by the plants in less than a week.

The difference of the means of the amounts of potassium in the tissues between the H-K and L-K cultures was statistically significant (odds 9999 : 1).

Potassium accumulated in the old (B) and mature (C) leaves of the H-K cultures in both series. The difference in the potassium content of the leaves of the H-K cultures between the N-n and A-n series was not as great between the H-K and L-K cultures and statistically less significant (odds 20 : 1), indicating that NH_4^+ had not greatly interfered with the absorption of K^+.

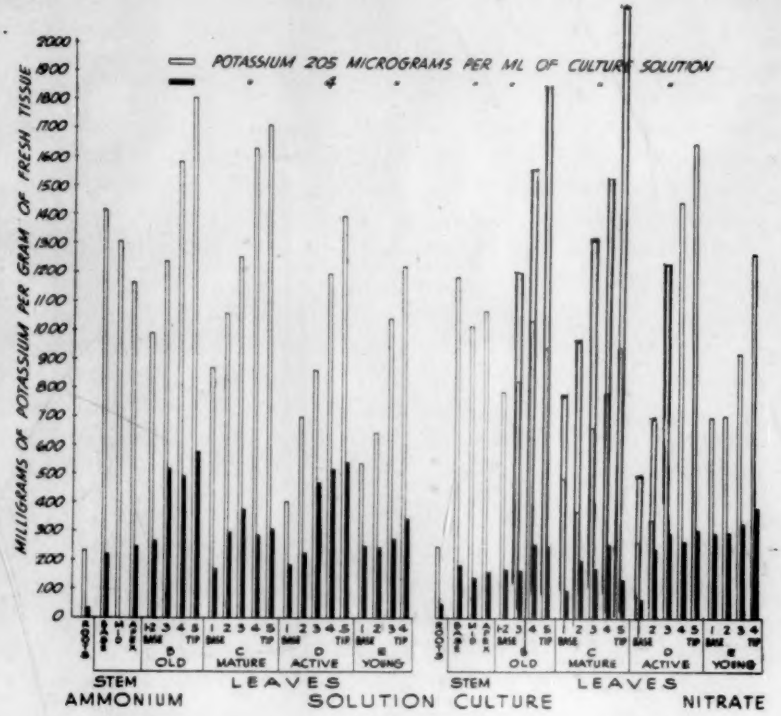

FIG. 2. Potassium content of pineapple tissues, fresh weight.

Potassium as percentage of total ash, calculated from the data in table V, was 73.0 and 86.0 per cent. in the H-K cultures for the N-n and A-n series, respectively, while in the L-K cultures the values were 32.0 per cent. for the N-n series and 50.4 per cent. for the A-n series. Figure 7, reporting concentrations of K in the roots, stem and leaves, calculated from the data in table V, shows that the leaves except in the H-K cultures of the A-n series, had higher K values than the other organs. Figure 8, reporting ratio values of stem to root K and of stem to leaf K shows that the leaves of the L-K cultures contained much greater amounts of K in their relationship to stem or roots than the leaves of the H-K cultures.

The stems of the A-n series contained more potassium than those of the

N-n series, possibly indicating a slightly lower rate of translocation from stem to leaves in the former series.

Calcium

The absorption of calcium was antagonized strongly by potassium as shown in table V and figure 3. Calcium absorption was also antagonized by ammonium ions. The combined or individual antagonism of potassium and ammonium on calcium absorption can be clearly seen by comparing the calcium content of plants in the A-n with that in the N-n series. The lack of antagonism by potassium in the L-K cultures increased the calcium con-

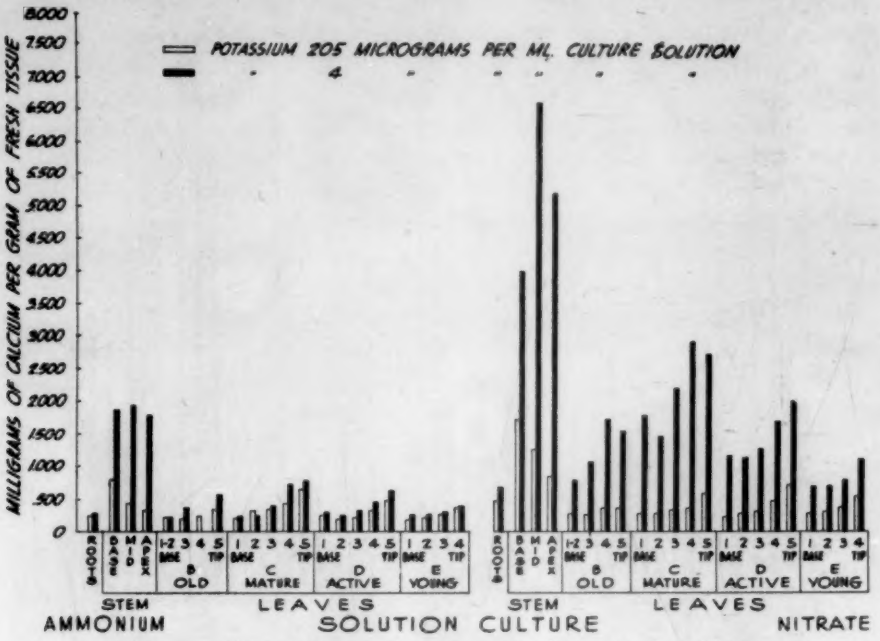

Fig. 3. Calcium per gram of pineapple tissues, fresh weight.

tent in these plants up to 6 times that in the H-K cultures although both cultures were supplied with equal amounts of calcium in the nutrient solution. The antagonistic effects of ammonium were of approximately the same magnitude since the plants of the N-n series contained up to 4 times as much calcium as those of the A-n series. Calcium accumulated mostly in the stem, the concentrations being from 2 to 6 times as great as in the leaves. It is possible that the higher calcium content of the stem reveals in the A-n series a low rate of translocation from stem to leaves.

Calcium as percentage of total ash, calculated from the data in table V, was 3.0 and 2.4 per cent. in the H-K cultures for the N-n and A-n series, respectively, while in the L-K cultures the values were 21.4 for the N-n and 7.4 per cent. for the A-n series. The L-K cultures contained 7.1 times more calcium in the N-n series and 3.1 in the A-n series than the H-K cultures,

indicating a greater rate of absorption of calcium from the nutrient solution in the presence of insufficient amounts of K. Figure 7 reporting concentrations of Ca in the roots, stem and leaves, calculated from the data in table V, shows that the stem contained higher values than the other organs. Also, figure 8, reporting stem to root Ca and stem to leaf Ca, shows that Ca was higher in the stem than in the other organs and that its movement from stem to leaves was greater in the H-K than L-K cultures.

Magnesium

Magnesium absorption from the nutrient solution as indicated by the magnesium content of tissues was reduced by the high concentrations of

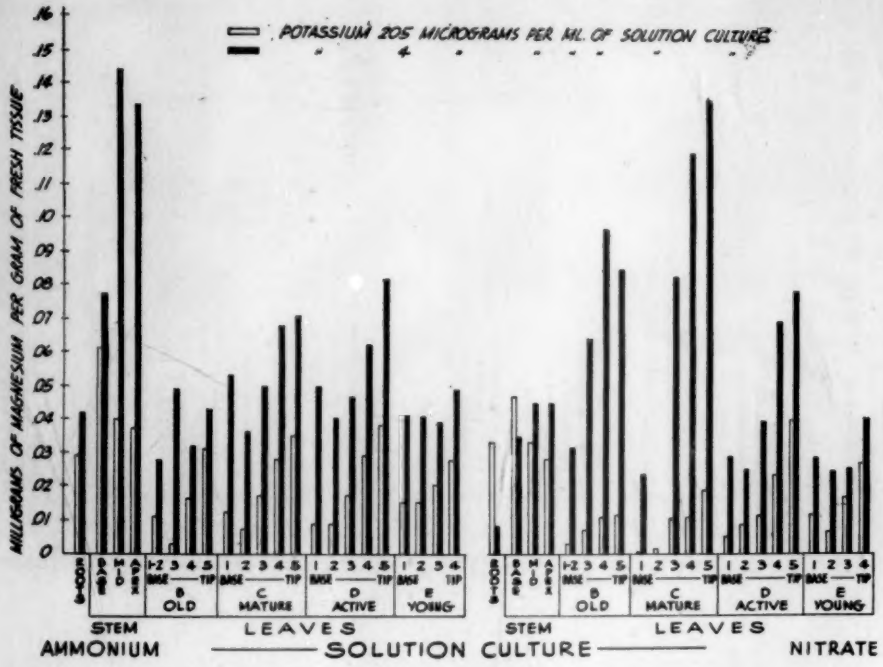

Fig. 4. Magnesium per gram of pineapple tissues, fresh weight.

potassium in the H-K cultures, as reported in table V and depicted in figure 4. However, the differences between H-K and L-K cultures in the magnesium content of tissues were not as great as those in the calcium content. With the exception of the mature (C) leaves of the L-K cultures in the N-n series the plants in the A-n series contained more magnesium than in the former series. Hence, the antagonistic effects of ammonium ions which were found to retard the absorption of calcium in the A-n series had not interfered in the absorption of magnesium.

Magnesium as percentage of total ash, calculated from the data in table V, was 1.17 and 1.93 per cent. in the H-K cultures for the N-n and A-n series, respectively, while in the L-K cultures the values were 7.60 per cent. for the

N-n and 8.42 per cent. for the A-n series. The L-K cultures contained, respectively, 6.50 and 4.36 times more magnesium for the N-n and A-n series than the H-K cultures, indicating a greater rate of absorption of Mg by the L-K than H-K cultures. Figure 7, reporting concentrations of Mg in the roots, stem and leaves, calculated from the data in table V, shows that the stem in all cultures, except in L-K of the N-n series, had higher values than the other organs. The ratio values of stem to root Mg and stem to leaf Mg in figure 8, calculated from the values in figure 7, show that Mg moved at a greater rate from the stem to the leaves of the L-K cultures in the N-n series than to the leaves of the other cultures.

The rate of magnesium accumulation in the stem, or translocation from stem to leaves, was different from that of calcium and almost reversed with

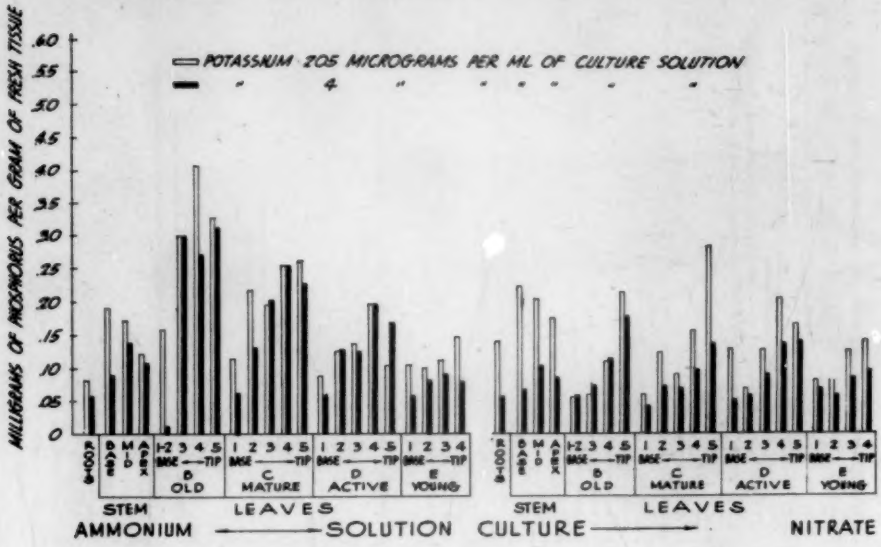

FIG. 5. Phosphorus per gram of pineapple tissues, fresh weight.

respect to the amounts of potassium and source of nitrogen in the nutrient solution.

PHOSPHORUS

The phosphorus content of the leaves was greater in the H-K than L-K cultures, as reported in table V and depicted in figure 5, and with respect to the source of inorganic nitrogen it was greater in the A-n than N-n series. The statistical significance of the difference of the means was high (odds 9999 : 1).

Phosphorus as percentage of total ash, calculated from the data in table V, was 0.895 and 1.40 percentage in the H-K cultures for the N-n and A-n series, respectively. In the L-K cultures the percentage values were 1.35 for the N-n and 2.22 for the A-n series. The differences in percentage values between the H-K and L-K cultures were small compared to those obtained

for calcium or magnesium. The concentrations of P in the roots, stem, and leaves, calculated from the data in table V and reported in figure 7, were higher in the leaves of all cultures except in the H-K of the N-n series. Also, the ratio values of stem to root P and stem to leaf P, in figure 8, show higher phosphorus in the leaves of all cultures except in the H-K of the N-n series. The lower phosphorus content of the cultures of the N-n series may be attributed to the antagonistic effects of NO_3^- ions. The data indicate that phosphorus absorption was increased by high potassium concentrations in the nutrient solution.

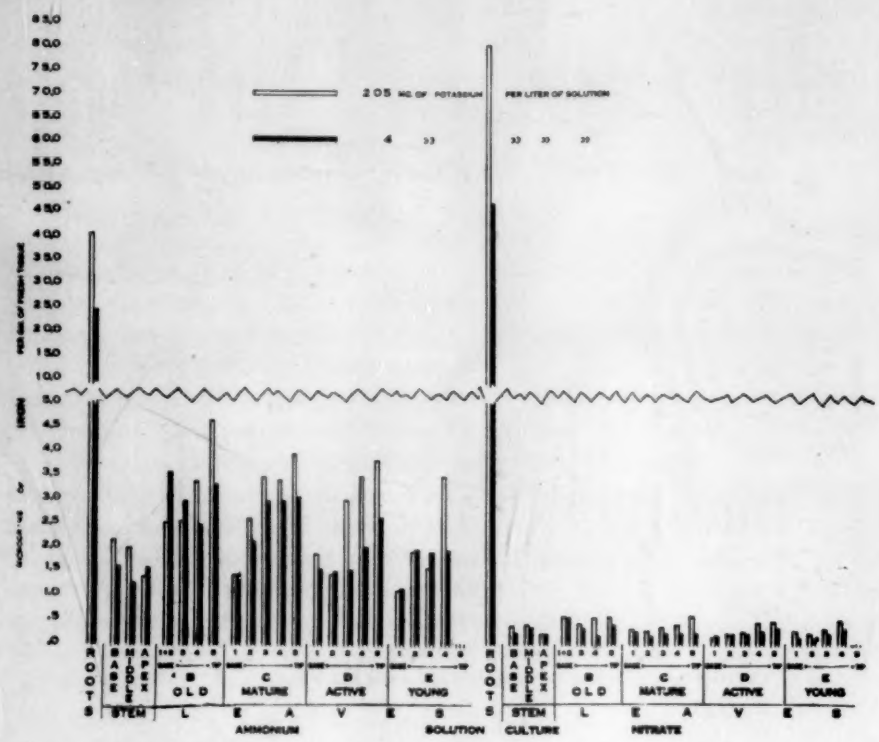

FIG. 6. Iron per gram of pineapple tissues, fresh weight.

IRON

Iron absorption by plant roots, as demonstrated by the iron content of tissues, was higher from the H-K cultures in both nitrogen series, as reported in table V and depicted in figure 6. However, iron absorption was increased in the A-n series as the result of a greater degree of solubility brought about by the high H-ion concentrations in the nutrient solution of this series. The greater accumulations of iron in the tissues of the H-K cultures had possibly resulted from a higher rate of metabolic activity which, in turn, increased the rate of absorption of this element. This supposition, supported by the greater amounts of iron in the roots of H-K than L-K cul-

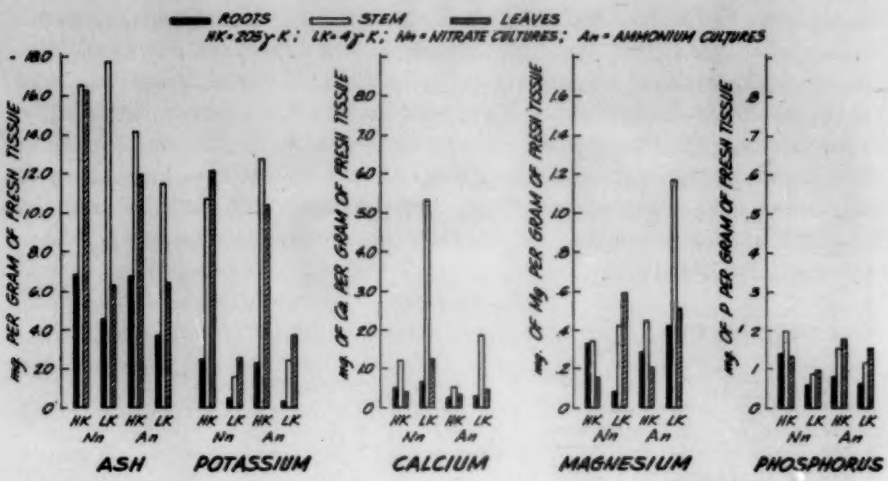
ROOTS
STEM
LEAVES
HK = 20 g K.; LK = 4 g K.; Nn = NITRATE CULTURES; An = AMMONIUM CULTURES
mg. PER GRAM OF FRESH TISSUE
mg. OF Ca PER GRAM OF FRESH TISSUE
mg. OF Mg PER GRAM OF FRESH TISSUE
mg. OF P PER GRAM OF FRESH TISSUE
HK LK HK LK
Nn An
ASH
POTASSIUM
CALCIUM
MAGNESIUM
PHOSPHORUS

FIG. 7. Mineral composition of one-year-old pineapple plants.

tures, suggests that iron was attracted to the root surface by electrostatic or comparable physico-chemical forces generated within the cells almost proportional to the metabolic activity of such cells, as indicated by the greater growth of the H-K plants. Iron was more abundant in the roots of the N-n than A-n series and conversely the leaves of the A-n series contained more iron than leaves of the N-n series. The differences between the iron content of leaves and roots in both series had possibly resulted from differences in iron solubility on the root surface on account of different pH values either on the root surface proper or in the nutrient solution. The higher pH values in the N-n than A-n series caused a greater deposition of insoluble iron on the roots of the former series which, consequently, lowered the degree of availability and absorption of this element and the rate of its translocation

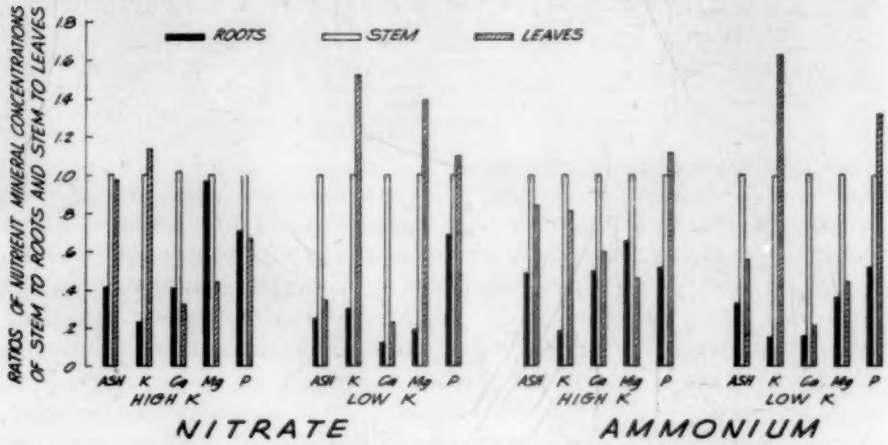

FIG. 8. Stem/root and stem/leaf ratios of pineapple plants in high- and low-potassium cultures.

from roots to leaves. Conversely, lower pH values in the A-n than N-n series decreased the amounts of insoluble iron deposited in the roots of the former series and also increased its absorption and translocation from roots to leaves.

Iron in the leaves as percentage of total iron, calculated from the data in table V, was 4.1 and 3.5 per cent. in the H-K and L-K cultures, respectively. In the A-n series the values were 50.95 per cent. for H-K and 33.97 per cent. for the L-K cultures. On the basis of these values the A-n series contained 12.5 and 9.6 times more iron in the leaves of the H-K and L-K cultures, respectively. The iron content of the leaves was higher in the H-K than L-K cultures, being 46.6 and 45.0 per cent. for the N-n and A-n series, respectively, indicating that percentage absorption as influenced by potassium was relatively uniform in both series.

Discussion

Increases in plant weights of *A. comosus* with higher potassium levels are in agreement with the results reported by PHILLIPS *et al.* (**30**) and NIGHTINGALE *et al.* (**27**) for tomato; SMITH and BUTLER (**45**) for wheat, corn and buckwheat; WHITE (**50**) for Lemna; GREGORY (**9**) for barley; JANSSEN and BARTHOLOMEW (**18**) for oats; and HARTT (**12**) for sugar cane.

The reports in the physiological literature on the water content or succulence of tissues from plants supplied with different amounts of potassium are not consistent. GREGORY (**9**), quoting SHIH (**38**), states that the water content of potassium-deficient plants depends on the levels of sodium, calcium and phosphorus, the phosphorus content having little effect on the water content at high potassium levels, whereas reduction of potassium resulted in increased water content much more at high than at low phosphorus levels. The same author (**9**) reporting on two types of potassium deficiency claims that the first type (high sodium, low calcium) is associated with increased succulence and the second type (high calcium, low phosphorus), with decreased succulence or low water content. In more recent studies, RICHARDS and SHIH (**34**) state that the water content increases markedly with potassium deficiency at the high sodium level, less markedly at medium sodium level in presence of medium calcium supply, while at high calcium level there was a minimum of succulence. They (**35**) state that highly significant positive correlations between phosphorus and water contents were found. JAMES (**17**) states that the leaf water content of potato plants showed no significant response to potassium, while PENSTON (**29**) claims that maximum potassium in potato coincided with maximum dry weight, water content, and total residual ash. MASON and PHILLIS (**23**) studied the effect of potassium on the hydration of cotton plants exposed to short and long daylight length and claim that in the long-day plants increasing potassium supply was accompanied by a reduction in water, while in the short-day plants there was an actual increase in water. They also found (**24**) that the salt content of disks cut from leaves showed a close rela-

tionship with water uptake and suggest that salt increases the hydration capacity of the leaf. The transpiration rate of plants in nutrient solutions lacking potassium decreases after an interval of time, according to SNOW (**46**). The greatest was that of potassium-deficient plants and occurred during periods of highest light intensity and temperature.

In *A. comosus* the differences in the water content of the tissues between the H-K and L-K cultures, although small, were in favor of the H-K cultures. The composition of the low potassium nutrient solutions, shown in table I, was a mixture of high sodium, medium calcium, and low phosphorus and from this point of view did not conform in every detail to either type of nutrient solution mentioned by GREGORY (**9**).

The increased salt and water contents of the leaf tissues of *A. comosus* in the H-K cultures are in satisfactory agreement with the views of PENSTON, but differ in some respects with those of the other investigators mentioned above. The higher water content of the stem sections in the L-K cultures resulted from the deposition of low amounts of starch rather than from any other cause as will be shown in a future publication.

Most investigators (**10, 13, 27, 30, 32, 45**) have found the potassium content of plant tissues proportional to the supply to the roots, which is in agreement with the findings in this study.

The effects of different amounts of potassium on the calcium, magnesium and phosphorus content of plant tissues have been studied by many workers with more or less similar results as reported herein. PHILLIPS *et al.* (**31**), MCCALLA and WOODFORD (**25**), BECKENBACH *et al.* (**3**), FONDER (**8**), HARTT (**12**), SCHMALFUSS (**37**) and OPPENHEIMER and MENDEL (**28**) have reported lower tissue calcium in cultures supplied with high than low potassium. LUNDEGARDH (**21**) explains the antagonistic phenomena of potassium on calcium absorption as follows: "Potassium in high amounts exerts a degenerative influence on the protoplasmic membrane of the roots by decreasing the native negative potential below – 50 mv. This lowering of the potential makes the protoplasm more liquid by intensifying its hydration and decreasing the H-ion concentration of the surface layer while calcium and magnesium act in the opposite direction—they dehydrate the membrane by attracting water molecules from the surrounding membrane area, which process increases the density, surface tension and stability of the membrane and its tolerance to higher electrostatic charges." SPIEGELMAN and REINER (**47**), having tested mathematically the various physical and chemical theories attributable to potassium transport and accumulation in animal tissues, are of the opinion that, on the basis of energy requirements, chemical as distinguished from electrical forces provide a satisfactory explanation of K movement in tissues. The association of chemical rather than physical forces with K movement and accumulation is also correlated with the polymerization functions of K in sucrose, starch, and protein synthesis. This will be presented in subsequent publications.

The results of the antagonistic effects of potassium on calcium as reported herein are in satisfactory agreement with those in the literature. LUNDE-

GARDH's assumption, however, that potassium in high concentrations produces degenerative effects on the protoplasmic layer of root cells does not harmonize with all observed data. For instance, in the H-K cultures where the amounts of potassium supplied to the roots exceeded 2 to 3 times those that could be absorbed, root cell injury would have been unavoidable had such degenerative effects been induced. Instead, the roots of the H-K cultures in the nitrate series weighed more than those of the L-K. The lower weights of the roots of the H-K than L-K cultures in the A-n series should be attributed to high H-ion rather than high K-ion concentrations. LUNDEGARDH's hypothesis on protoplasmic degeneration may have some relation, however, to certain other phenomena (unpublished studies) where the meristematic apical tissues of the stem of plants supplied with 200 mg. of K and 160 mg. of N per liter of nutrient solution had failed on account of cell injury to continue normal growth and produce a fruit but formed lateral vegetative proliferations, a rare phenomenon for *A. comosus*.

The phosphorus content of tissues was higher in the plants supplied with high- rather than with low-potassium. BARTHOLOMEW *et al.* (**2**) observed in the tomato plant that an abundant supply of potassium increased the absorption of phosphorus whereas the opposite is claimed by JOHNSTON and HOAGLAND (**19**) and WALL (**49**).

The data of JOHNSTON and HOAGLAND (**19**) and WALL (**49**) for tomato and HARTT (**12**) for sugar cane showing higher phosphorus in minus-K or low-K than in high-K cultures are in disagreement with the findings of BARTHOLOMEW *et al.* (**2**) for tomato and of the authors for *A. comosus* who observed the opposite condition. It is possible, however, that the disagreement had resulted from differences in the chemical composition of the nutrient solutions employed by these investigators. The phosphorus content of the nutrient solutions, expressed as mg. per liter, was 279 in WALL's, 139 in HARTT's and 15 in the authors' experiments. Also, the K content of the plus-K cultures of Johnston and Hoagland and of the high-K cultures of the authors was different, being 39 and 205 mg. per liter for the former and latter investigators, respectively.

RICHARDS (**33**) claims that high amounts of phosphorus are toxic to potassium-deficient plants and that such toxicity may be counteracted by adding rubidium to potassium-deficient cultures. NIGHTINGALE (**26**), observing that certain relations between potassium, on the one hand, and phosphate and nitrate, on the other, modified the growth and fruiting habits of *A. comosus* grown under field conditions, claims that phosphates antagonize the absorption of nitrates and vice versa and that the decrease through mutual antagonism of either one during certain critical stages of plant development may cause reduction in plant growth and fruit yields by a curtailment of the carbohydrate reserves, presumably on account of either rapid utilization by high nitrate absorption or improper assimilation by insufficient phosphate intake.

The greater iron content of the plants in the H-K than L-K cultures was attributed to a higher rate of growth, water absorption and metabolism.

ROHDE (**36**) states that potassium-deficient plants contain low amounts of iron but high amounts of manganese. Moreover, he asserts that catalytic enzymes containing iron which influence assimilation either decrease or completely cease their activity in potassium-deficient plants, whereas enzymes containing manganese are able to continue their function in assimilation and, in consequence, promote certain metabolic changes which cease on account of iron deficiency. The greater amounts of iron found in potassium-deficient as compared with potassium-supplied plants of sugar cane (**12**) and corn (**15**) are explained as having resulted from an unspecified root injury.

The data herein reported and those in the literature show a satisfactory agreement between amounts of potassium supplied to the roots and plant weights, hydration, iron, potassium content of tissues, and intensity of antagonism to the absorption of calcium and magnesium. The results on the phosphorus content of tissues were not in agreement with the findings of all other investigators and this disagreement has been attributed primarily to differences in the composition of the nutrient solutions. However, differences ascribable to the distant familial relationships, as between *Ananas comosus, Zea mays* and *Saccharum officinarum*, should not be overlooked. GREGORY and CROWTHER (**9, 11**), having studied five varieties of barley, claim that they may be characterized by their mineral nutrient efficiencies. LYNESS (**22**) states that three varieties of corn, compared in their capacity for absorbing phosphates from sand cultures of similar concentration, were in the ratio of 3:5:7. Many of the disagreements may be justified either by differences in familial plant relationship or by differences in composition of nutrient solutions.

Antagonistic effects by ammonium on calcium were observed by STEWART, THOMAS, and HORNER (**48**) and SIDERIS, KRAUSS, and YOUNG (**44**) on *A. comosus;* BURSTRÖM (**4**) on hops; and ASPREY (**1**) on dices of potato tubers.

Summary

The effects of high versus low amounts of potassium on the growth and ash constituents of *Ananas comosus* (L.) MERR., in solution cultures supplied with equal amounts of nitrogen from nitrate or ammonium salts were as follows.

1. The plants of the high-potassium cultures in the nitrate series weighed 40 per cent., and in the ammonium series, 73 per cent. more than those of the low-potassium cultures. The differences in stem weights between the high- and low-potassium cultures were much greater than those of plant weights; being greater for the high- potassium cultures, 94 per cent. in the nitrate and 185 per cent. in the ammonium series.

2. Ash values and succulence, the latter as measured by the water content of tissues, were generally higher in the high- than in the low-potassium cultures.

3. Potassium was from 2 to 7 times higher in the tissues of the high- than of the low-potassium plants.

4. The calcium and magnesium content of tissues was many times higher in the low- than in the high-potassium plants. The calcium content of the tissues was appreciably lower, however, in the plants supplied with ammonium- than with nitrate-nitrogen. The phenomena of antagonism between potassium on the one hand and calcium and magnesium of the other are discussed.

5. Phosphorus was more abundant in the high- than in the low-potassium plants. The phosphorus content of the plants in the ammonium series was greater than in the nitrate series.

6. The plants in the high-potassium cultures contained more iron than in the low-potassium cultures. Also, those of the ammonium series contained more iron than of the nitrate series, indicating that the higher H-ion concentrations in the ammonium series had favored the absorption of iron by increasing its solubility.

7. The results are compared with those of other workers, the differences are indicated and their significance is discussed.

The assistance of H. H. Q. CHUN is gratefully acknowledged.

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QUANTITATIVE STUDY OF ETHYLENE PRODUCTION IN APPLE VARIETIES[1]

ELMER HANSEN

(WITH THREE FIGURES)

The capacity for ethylene formation is present in many kinds of fruits but appears to be entirely absent in others. In apples and pears the amounts of ethylene produced vary greatly between varieties; according to the available data, the intensity of production is correlated with the longevity of the fruit in storage. In six varieties of apples, NELSON (**5**) found that those with the longest storage life were characterized by the least capacity to produce ethylene. Similarly in pears, the short-lived variety, Bartlett, was observed to have a maximum rate of ethylene production six to seven times greater than the long-lived variety, Anjou (**2**). The formation and accumulation of ethylene in the fruit tissues is a vital factor in the natural ripening processes of pears (**3**) and probably of apples (**4**). In the present study, the production of ethylene by apple varieties in relation to season of maturation, rate of ripening, length of storage, respiration, and temperature are considered.

Materials and methods

Five varieties of apples differing in time of maturity from mid-summer to late fall were obtained from the Experiment Station orchard located near Corvallis. The samples were carefully selected for uniformity in size, maturity, and freedom from blemishes. The summer-maturing apples (Astrachan, Red June, and Gravenstein) were picked when firm but partially colored and placed immediately in the respiration chambers maintained at 20° C. (68° F.). The fall-maturing varieties (Delicious and Newtown) were harvested on September 23 and October 1, respectively, packed in oiled paper wraps, and stored at 0° C. (32° F.). Samples were withdrawn from storage at various intervals for ethylene and respiration measurements at 20° C. and similar determinations were made at 0° C. Due to the development of bitter pit in the Newtown apples, investigations on this variety were discontinued after February 12.

The rates of ethylene and CO_2 production were determined simultaneously on the same samples of fruit, according to the methods described previously (**2**).

Results

EARLY-MATURING VARIETIES

Astrachan, Red June, and Gravenstein apples mature and ripen consecutively during the summer in the order listed. According to the data (fig. 1)

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there is an inverse correlation between the length of the maturation period of a variety and the amount of ethylene produced during ripening. Thus, the rate of ethylene production (ml. per kgm./24 hrs.) was highest in Astrachan (11.39 ml.), intermediate in Red June (9.27 ml.), and lowest in Gravenstein (5.15 ml.). With the exception of Astrachan, the amounts of ethylene produced on the first day following picking were below the chemically-determinable minimum (0.001 ml.) but increased rapidly to maximum values during an 8–10-day period. The peak in ethylene production was reached 4–5 days later than that for CO_2 production. NELSON (**6**) reported similar results for McIntosh apples; in pears, however, the maxima for

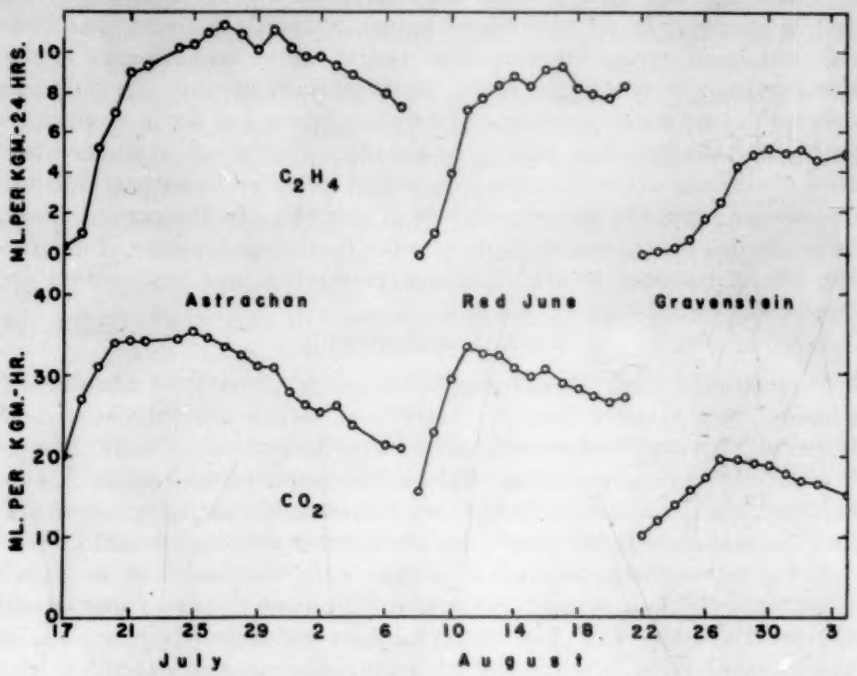

FIG. 1. Production of ethylene and respiration in three varieties of apples which mature and ripen at intervals during the summer season.

ethylene and CO_2 production were found to coincide (**2**). The relative increase in production during the climacteric was much greater for ethylene than for carbon dioxide. In Astrachan apples, ethylene production rose from 0.040 ml. to 11.38 ml. per kgm./24 hrs., approximately a 30-fold increase; while CO_2 production rose from 20.09 to 35.32 ml. per kgm./hr., less than a 2-fold increase. In Red June and Gravenstein, the comparative differences were even greater. This lack of correlation between ethylene and CO_2 production would appear to indicate that several oxidation-reduction systems function in the respiration of apples; probably only one of these is associated with the formation of ethylene. If the total CO_2 of respiration as well as ethylene were formed by the activity of a single respiratory enzyme

system, then a more definite quantitative relationship should exist between these two products than has been observed in these and former experiments (2).

LATE-MATURING VARIETIES

The Delicious and Newtown apples (Figs. 2, 3) maintained a much lower rate of ethylene production during ripening than the early-maturing varieties. There was very little difference between the amounts of ethylene produced by Delicious and Newtown, but the rate of production increased much slower in the latter variety. In the Delicious apples ripened after 4 weeks of storage, the rise in ethylene production was completed in 14 days but in

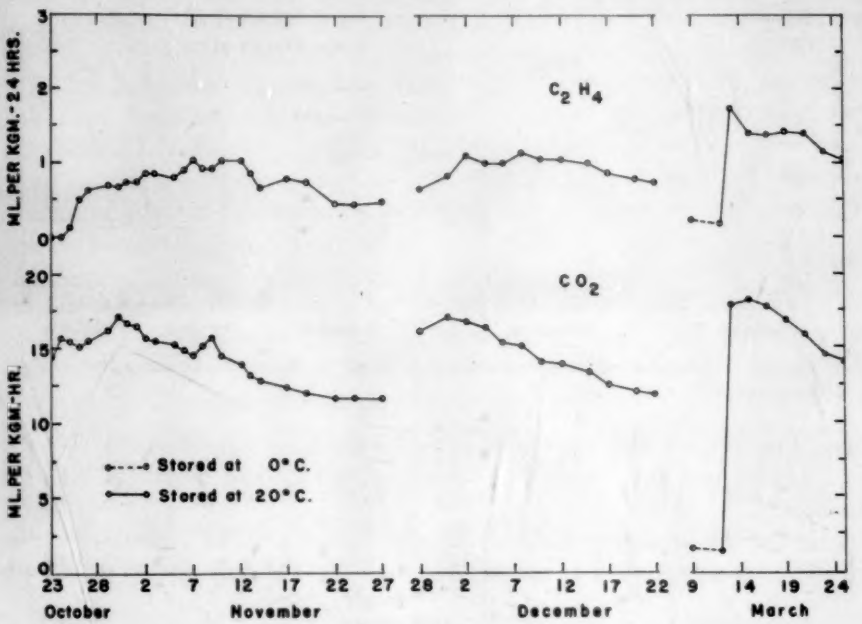

FIG. 2. Production of ethylene and respiration in Delicious apples after various storage intervals.

Newtown extended over a period of 40 days. The intensity of ethylene production evidently increases at cold temperatures, since lots of fruit withdrawn at later dates showed increasingly higher initial rates of ethylene production. This increase is undoubtedly associated with a steady progress in ripening of the fruit during the storage period.

The rates of ethylene production by Delicious, Newtown, and Jonathan apples during storage at 0° C. are shown in table I. These three varieties were not at the same stage of ripeness when the determinations were made; hence the results are not comparable on a varietal basis. According to these data, apples at 0° produce ethylene at approximately one-eighth to one-eleventh the rate at 20° C. Under commercial conditions, it would be possible for considerable ethylene to accumulate in the cold storage rooms, since

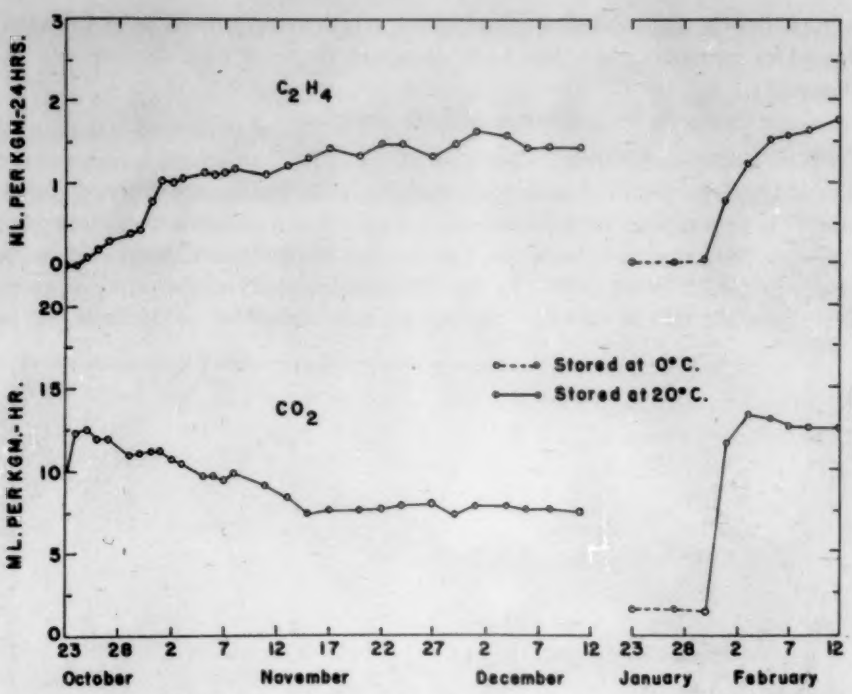

FIG. 3. Production of ethylene and respiration in Newtown apples after various storage intervals.

according to these data one bushel of apples produces approximately 1–4 ml. of ethylene per day, depending upon the variety and length of time in storage.

Neither Delicious nor Newtown apples showed a distinct climacteric rise in CO_2 production, such as occurred in the early-ripening varieties. Respi-

TABLE I

CARBON DIOXIDE AND ETHYLENE PRODUCTION IN APPLES AT 0° AND 20° C.

DATE, 1944	TEMPERATURE	ML. C_2H_4/KGM.-24 HR.	ML. CO_2/KGM.-HR.
	°C.	ml.	ml.
Delicious			
March 6– 9	0	0.223	1.78
March 9–12	0	0.198	1.46
March 12–13	20	1.770	18.03
Newtown			
Jan. 23–27	0	0.061	1.69
Jan. 27–30	0	0.082	1.54
Jan. 30–Feb. 1	20	1.770	11.85
Jonathan			
Feb. 2– 5	0	0.185	2.20
Feb. 5– 8	0	0.184	2.27
Feb. 8–10	20	1.590	18.15

ratory activity in the first samples ripened increased slightly during the first few days then declined during the remainder of the period. This behavior is especially apparent in the Newtown apples and is opposite to the trend in ethylene production. A more distinct climacteric rise in respiration may have been observed at time of harvest, although this is questionable in view of the results reported by EZELL and GERHARDT (1).

Summary

The production of ethylene and the respiration in five varieties of apples ranging in season of maturity from midsummer to late fall have been studied. Early-maturing summer apples were found to produce more ethylene during ripening than late-maturing fall varieties. The maximum rates of ethylene production at 20° C., expressed as ml. per kgm./24 hrs., were as follows: Astrachan, 11.38; Red June, 9.27; Gravenstein, 5.16; Delicious, 1.77; and Newtown, 1.78 ml. With the exception of Astrachan, the amounts of ethylene produced by mature but unripened fruit were below the sensitivity of the analytical method used (0.001 ml.). The rates of ethylene production increased rapidly after picking in the summer-maturing apples but slowly in Delicious and Newtown.

Apples stored at 0° C. produced ethylene at approximately one-eighth to one-eleventh the rate at 20° C. The rates of production at 0° C. for Delicious, Newtown, and Jonathan were 0.211, 0.071, and 0.185 ml. per kgm./24 hrs., respectively. The data obtained indicate that the intensity of ethylene production increases during the storage period.

Early-maturing varieties of apples showed a distinct climacteric rise in respiration, but this was not apparent in either the Delicious or Newtown apples. The increase in ethylene production during ripening does not appear to be correlated with total CO_2 production.

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OBSERVATIONS ON THE CAUSES OF THE FLOW OF SAP IN RED MAPLE

CLARK L. STEVENS AND RUSSELL L. EGGERT

(WITH ONE FIGURE)

Introduction

Sap flow in the maples occurs in great profusion under conditions and in a season during which few other species of plants exhibit this phenomenon. It is known to vary from year to year, from place to place, and from tree to tree, yet everywhere it follows the same general pattern. In many of its characteristics it does not seem to conform to the theories advanced to account for its occurrence. It cannot be ignored, yet it never has been satisfactorily explained.

Over a period of many years, a number of theories have been advanced to account for the mechanism by means of which water is moved to the tops of plants. Most of the experiments have been done on short lengths of excised tissue, or on herbaceous plants of no great height, and at a time when those plants were in leaf. Few data have been presented to account for the rise of sap in maple trees in early spring, or for the reason that such large quantities of sap can be collected from trees of certain species when tapped at that season of the year.

A review of literature on the subject indicates that more than one mechanism may be responsible for movement of water to the top of the plants. The "root pressure," the "vital" or living cell, and the "cohesion of water" theories have been advanced as forces individually capable of doing the work. For an extended discussion of each of these see MEYER and ANDERSON (**8**). They state that most plant physiologists agree that the cohesion theory explains the principal mechanism involved in the transportation of water, while the root pressure and living cell theories are of minor importance. The investigations of CLARK (**1, 2**) in Massachusetts, JONES and his co-workers (**3, 4, 5, 6**) in Vermont, and MORSE and WOOD (**9, 10**) in New Hampshire are more directly concerned with the characteristics of sap flow in maple trees.

Plan of the investigation

LOCATION

The investigations described below were carried out in a stand of mixed hardwoods, 20 to 40 years old, in the University Forest at Durham, New Hampshire. This is in the southwestern part of the state, about 15 miles inland from the coast.

The stand is composed principally of red maple, mixed with various other species, such as red oak, aspen, basswood, grey birch, paper birch, white elm, black cherry, sugar maple, white pine, and hemlock. It is fairly dense,

approximately eight-tenths of full stocking. The trees average 40 feet in height with rather narrow crowns. They are located on the southwest slope of a glacial ridge which rises to about 100 feet above sea level. The top of the ridge is planted to an apple orchard and the hardwood stand is situated part way down the hill. The trees studied are close to the edge of the orchard. The gradient here is about 5 per cent. and the drainage is into the stand. There is some sheet erosion, especially during the spring, and the trees have obviously derived a considerable supply of mineral salts from the cultivated area above them. The soil is a stony clay loam and, while there is a plentiful supply of moisture, drainage is evidently satisfactory.

The climate here may be termed moderate. The mean annual temperature is about 45° F. and the average rainfall is approximately 36 inches. Precipitation is fairly evenly distributed throughout the year. Weather conditions during the winter of 1943–44 were not usual for the locality. Snow, which arrived in January, was preceded by low temperatures in December, during which time the ground froze to a depth reported to be as much as 29 inches in some parts of Durham. On March 11, a soil well, dug in the vicinity of the experimental trees, showed two inches of ice on top of the ground and 12 inches of frozen soil below it. Very little snow, not over six to eight inches, was present on the ground for any length of time during the winter.

The trees selected for the experiment were red maples, *Acer rubrum* L., ranging from 6 to 9 inches in diameter breast high, and with crowns of approximately equal development.

Methods

During the month of December, 1943, thermocouples were installed in the experimental trees just inside the bark in the cambium region, in the roots four inches below the soil surface, and in the trunk at points six inches, five feet and 18 feet above ground. They were also located in the soil at the side of the roots, and in the centers of trees one foot above the ground. Lead-wires passed through pipes to the interior of a small cabin which could be heated to provide a suitable temperature for the operation of the potentiometer. A distance thermograph of the capillary tube type was installed in an instrument shelter, with the bulb inserted in the center of one of the control trees. The opening was sealed with grafting wax. A second thermograph was set up to record temperatures at a depth of from six to ten inches in the soil near the roots of this tree, and a third to record the temperature of the air. All three were housed in the same instrument shelter.

On February 25, experimental tree no. 1 was severed at about five feet from the ground, using a saw and axe to make a smooth cut. By means of a block and tackle the tree was lowered immediately into a half-barrel filled with water. Guy wires attached to the crown kept the tree in an upright position. Only a small amount of sap oozed from the saw kerf when the tree

was cut. The stump also stopped flowing, and the cut surface dried in a few minutes after the tree was severed. What little sap there was exuded came from the south side of the tree.

Experimental tree no. 2 was pruned of all branches and the tip. All cuts were sealed immediately with grafting wax. Tree no. 3 was severed at about five feet from the ground, after all limbs and the tip had been removed, and the limb wounds sealed with grafting wax. The trunk, which was about 37 feet tall, was set upright in a second tub of water. Tree no. 4 was cut into five eight-foot sections, the branches removed, and the top of each section and all wounds were sealed. Counting from the butt, sections 1, 3, and 5

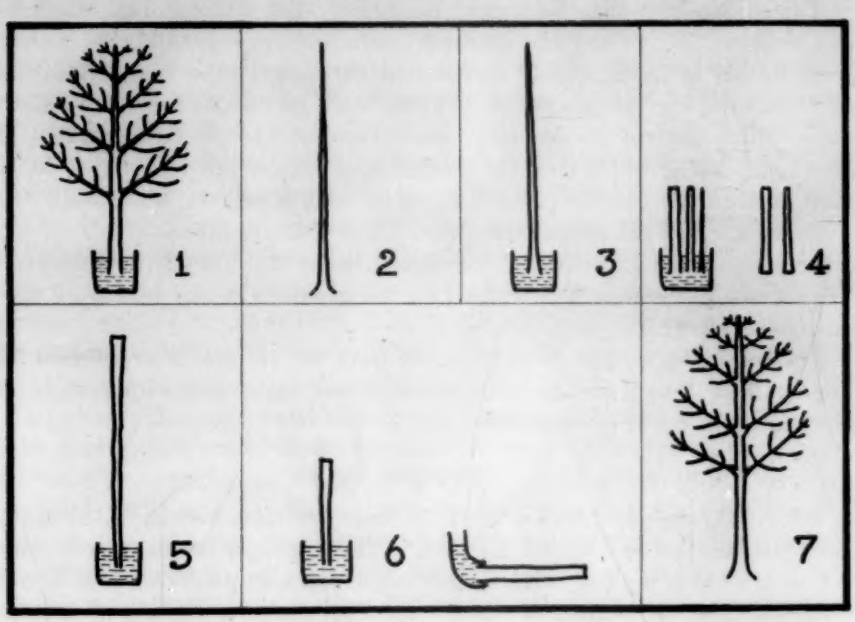

FIG. 1. Diagrams illustrating treatment of experimental trees.

were set upright in a tub of water. Sections 2 and 4 were set upright on a dry base with both ends of each section sealed with grafting wax.

Tree no. 5 was severed from the stump; all limbs and the tip were removed to a height of 24 feet, after which it was set in an inverted position in a tub of water. The limb and basal cuts were sealed. Two eight-foot sections were taken from tree no. 6. The lower one was placed in a horizontal position and the smaller end sealed with grafting wax. A piece of rubber inner-tube, which was kept filled with water, was attached to the larger end. The second eight-foot section was set in a tub of water in an inverted position. See figure 1 for diagrams illustrating the treatment given the experimental trees.

As to the controls, tree no. 7 was the one with the thermograph bulb installed in it, tree no. 8 had thermocouples at various points in the cambium

layer, while tree no. 9 had no devices for measuring temperatures. Each of the nine trees, except no. 8, was tapped at approximately four and one-half feet above ground, and some at other heights. Sap flowing from the spouts was tested in a refractometer and, in some cases, the quantity of flow was recorded.

Results

In practically every case, sap of normal quality flowed as consistently from the spouts in the experimental trees as it did from those in the control trees. The few exceptions are noted below.

Tree no. 1 (severed) was tapped a second time, at about seven feet above ground and a broken branch was observed at about 30 feet from the ground. Sap flowed freely from all three openings. Twelve daily tests of sap from the lower spout showed an average sugar content of 2.91 per cent. An average of eight tests of the sap from the second spout showed a content of 3.2 per cent. These figures are based on tests made with the refractometer. While they are doubtless somewhat higher than the actual sucrose content, they will serve to indicate the relative sweetness of the sap from the various spouts. They should not be interpreted, however, as reliable expressions of the true sugar content.

Tree no. 2 (stripped trunk) had an open wound in the bark at about 30 feet from the ground. Although sap ran from this opening, it was not tested. Sap from the spout had a sugar content 3.7 per cent., an average of 17 tests.

Tree no. 3 (stripped and severed) was tapped a second time at a point 31 feet above ground. Eight tests of the sap from the lower spout showed a sugar content of 3.8 per cent. Sap was observed to flow from the upper spout on several occasions but was not tested.

Tree no. 4 was the tree cut in eight-foot sections. Four tests made on the sap flowing from section 1 (the butt) showed an average sugar content of 3.6 per cent. Section 3 did not flow as freely as the other two. Three tests showed a sugar content of 3.9 per cent. Section 5 (the tip) yielded sap testing 4.1 per cent., an average of four different tests. The two sections, set on a dry base, were tapped at about a foot from the upper end, and again at a foot from the lower end. Neither spout yielded enough sap to test. There was a little oozing of sap, however, around the spouts, so that the bark on the trunks was wet for some distance. After the experiment had run for some time, section 1 was removed from the tub early one morning, following a night when the temperature dropped to 28° F., and set in an empty bucket. Within four hours, 850 cubic centimeters of sap flowed out of the base, and tests of this showed a sugar content of 2.0 per cent. At the same time section 2 was removed from the dry situation, the sealed end was sawed off, and the stick was inserted in a tub of water and tapped at about four and one-half feet from the lower end. After a few days, sap flowed from this spout as freely as from the other sections in the tub, and a single test showed a sugar content of 5.2 per cent.

At the time this tree was cut, a short section was sawed from between each of the eight-foot sections as well as from the stump. These sections were taken to the Chemistry Laboratory of the Engineering Experiment Station where the sugar content was determined. Table I shows the results of these tests.

Tree no. 5 (stripped, severed, and inverted) was tapped a second time at a point 21 feet from the ground. The lower spout yielded sap testing 3.2 per cent., an average of six tests. Sap was observed flowing from the upper spout on two mornings only for a period of about one hour each, at a time when the flow from other trees was very profuse. No samples were saved for testing.

Tree no. 6 was cut into two eight-foot sections. The upper was placed upside down in a tub of water, and the lower was laid in a horizontal position with a piece of inner-tube stretched over, and sealed on, the butt end. This made it possible to keep the section constantly supplied with water.

TABLE I

SUCROSE CONTENT OF WOOD, TREE NO. 4

SECTION	HEIGHT ABOVE GROUND	SUCROSE EXTRACTED PER GRAM OF WOOD
	ft.	*gm.*
1	1	0.0260
2	9	0.0104
3	17	0.0052
4	25	0.0156
5	33	0.0260
6	41	0.0313

The inverted section in the tub was tapped at four and one-half feet from the base. Sap flowed from it, and four tests showed a sugar content of 3.2 per cent. The horizontal section was tapped in three places—the center, and two feet on each side of it. Sap flowed rather well from each of the three spouts. The one nearest the original base of the tree tested 1.6 per cent., an average of six tests. The spout in the center yielded sap testing 2.2 per cent., an average of six tests. Sap from the third spout tested 2.7 per cent., an average of four tests. After this section had been allowed to flow for some time, the inner-tube was removed and the water supply discontinued. Three days later sap still oozed from around the sap spouts and moistened the bark along the outside of the trunk. There was also a noticeable quantity of sap wetting the bark around the sealed upper end of the section.

Observations were made on the removal of water from barrels containing the severed trees, the stripped and severed trunks, and the sectioned trunks. For a period of seven days after the severed tree was placed in the barrel, accurate data could be obtained on the absorption of water. During the next period of two weeks, the temperature dropped to from 10° to 15° F. on several occasions. This resulted in the production of so much ice in the

TABLE II

TEMPERATURES AT DIFFERENT POINTS IN A SEVERED MAPLE TREE AND STUMP AND IN TWO CONTROL TREES DURING THE TIME WATER WAS BEING ABSORBED. AIR TEMPERATURE DROPPED RAPIDLY TO A LOW DEGREE

SATURDAY, MAR. 18, 1944	POSITION OF THERMOCOUPLES									THERMOGRAPH			
	SEVERED TREE, NO. 1				CONTROL TREE, NO. 8					TREE NO. 7			
	1	2	3	4	5	6	7	8	9	10	11	12	13
	CAMBIUM 18′ HIGH, SOUTH SIDE	CAMBIUM OF ROOT, 4″ DEEP IN SOIL	SOIL AT SIDE OF 2	CENTER OF STUMP, 1′ ABOVE GROUND	CAMBIUM 18′ HIGH, SOUTH SIDE	CAMBIUM OF ROOT, 4″ DEEP IN SOIL	IN SOIL AT SIDE OF 6	CAMBIUM 6″ HIGH, SOUTH SIDE	CAMBIUM 6″ HIGH, NORTH SIDE	CENTER OF TRUNK, 4′ HIGH	AIR TEMP.	BAROMETRIC PRESSURE	CONDITION OF WATER IN BARREL
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	in.	
8:30 A.M.	34.50	32.00	32.00	.....	42.50	31.50	31.50	32.50	32.50	34.0	37.00	29.65	
9:00 "	39.50	32.00	32.00	.....	46.50	31.50	31.50	38.00	34.75	34.0	39.50	29.66	
9:30 "	51.00	32.00	32.00	.....	47.00	32.00	32.00	42.00	36.00	35.0	41.00	29.67	
10:00 "	57.50	32.00	32.00	.....	46.50	32.00	32.00	42.50	37.50	35.0	42.50	29.67	
11:00 "	60.50	32.50	32.50	.....	52.75	32.00	32.00	49.50	40.00	35.5	45.00	29.67	
PARTLY CLOUDY; STRONG NORTH-WEST WIND; TEMPERATURE REMAINED AT 45° F. UNTIL 4:30 P.M.; DROPPED 23° IN FOUR HOURS													
8:30 P.M.	29.50	31.00	31.00	.....	28.00	31.00	31.00	29.50	28.50	39.0	24.25	29.87	$\frac{1}{16}$ inch of ice
8:50 "	27.75	31.50	31.50	.....	26.75	31.00	31.00	30.50	28.00	38.5	22.75	29.89	
9:05 "	27.75	31.00	31.00	38.00	26.50	31.00	31.00	30.00	29.00	37.5	22.25	29.90	
9:15 "	27.00	31.00	31.00	38.25	27.00	31.00	31.00	29.50	28.75	37.0	22.00	29.91	$\frac{1}{8}$ inch of ice; air bubble under it
9:25 "	27.75	31.50	31.50	37.00	27.00	31.00	31.00	29.50	29.00	36.0	21.50	29.93	
9:35 "	27.75	31.25	31.25	37.00	26.00	31.25	31.25	29.25	28.50	36.0	21.25	29.93	
9:45 "	28.00	31.50	31.50	36.75	26.00	31.00	31.00	29.50	28.75	35.5	21.00	29.95	1 liter of water absorbed from under ice
10:00 "	27.00	31.50	31.50	36.25	25.50	31.00	31.00	29.25	28.50	35.0	20.00	29.96	
10:10 "	27.50	32.00	32.00	35.50	25.00	31.50	31.50	29.00	28.50	35.0	19.50	29.97	
10:20 "	26.50	32.00	32.00	35.00	24.75	31.25	31.25	28.50	28.25	34.5	18.75	29.98	1.5 liters absorbed
10:30 "	26.25	32.00	32.00	34.75	24.25	31.25	31.25	28.75	28.00	34.0	18.25	29.99	
10:40 "	25.00	32.00	32.00	34.25	23.50	31.00	31.00	28.50	27.50	33.5	17.75	29.99	
10:50 "	25.00	32.00	32.00	34.00	22.50	31.75	31.75	28.00	27.25	33.0	17.00	30.00	2 liters absorbed
11:00 "	25.00	32.00	32.00	34.25	22.25	31.50	31.50	28.00	27.25	32.5	17.50	30.01	
11:10 "	23.75	32.00	32.00	33.75	22.00	31.25	31.25	27.50	26.75	32.0	16.50	30.02	2 liters absorbed

barrels that observations during the latter period were discontinued. After ice had entirely thawed (except for the small quantity which froze at the surface of each barrel every night on which the temperature was sufficiently low for it to form) additional data on absorption were obtained.

On cloudy days the temperature of the cambium did not rise above that of the air, but on clear days of bright sunshine cambium temperatures frequently went to 60° F. on the south sides of trees at mid-day, even though the temperature of the air did not rise much above 32° F. By late afternoon on those days, the centers of the trees registered temperatures ranging from 33° to 48° F.

Shortly after sundown, the temperature of the cambium would drop to within one or two degrees of the temperature of air, but a considerable lag occurred in the drop of internal temperatures of the trees. This is indicated in table III. Highest temperatures in the center of the tree were not reached until from 4:00 to 8:00 P.M. Ice would form at the surface of water in the barrels by the time air temperature had dropped to from 31° to 29° F. The exact point was difficult to determine since air temperature did not remain constant.

By the time $\frac{1}{16}$ to $\frac{1}{8}$ of an inch of ice had formed at the surface of the water in the barrels, the temperature of the cambium registered from 28° to 31.5° F. The temperature of the centers of the trees at this time was still from 33° to 38° F. While temperatures were in this relation air bubbles began to appear under the ice, and within two to four hours from $\frac{1}{4}$ to $1\frac{1}{2}$ inches of water (1 to 6 liters) moved out from under the ice and into the trees. The absorption of this water took place from barrels containing either stripped or unpruned tree tops. Tables II and III show the temperatures of different parts of the trees recorded on two different nights at the time water was being absorbed and the effects of the rate and amount of temperature fall on absorption. Neither of these nights would have been considered ideal for the absorption of water, since the temperature dropped too rapidly on March 18, and did not go low enough on March 31.

Tree temperatures were recorded on these two nights only. However, records of air temperatures were made by means of the thermograph. When water was removed from under the ice which formed at the top of each barrel the amount was measured early the following morning. From February 25 to April 11 this type of absorption occurred 19 times in quantities varying from a fraction of a gallon to over four gallons (less than 1 to over 15 liters). The amount absorbed varied according to the volume of the absorbing area and the rate at which the temperature of the air fell to some point below freezing. No water was removed from the barrels on nights when it did not freeze. This was indicated by lines drawn on the trunks at the water line the preceding evening.

On March 13, two main scaffold limbs of a 20-foot Delicious apple tree were cut off at the point where they were attached to the trunk. With branches intact they were placed in a barrel containing 25 gallons (95 liters)

TABLE III

TEMPERATURES IN THE SAME TREES AS THOSE OF TABLE II, BUT AT A PERIOD DURING WHICH AIR DID NOT GET AS COLD

FRIDAY, MAR. 31, 1944	POSITION OF THERMOCOUPLES									THERMOGRAPH			
	SEVERED TREE, NO. 1				CONTROL TREE, NO. 8					TREE NO. 7			
	1	2	3	4	5	6	7	8	9	10	11	12	13
	CAMBIUM 18′ HIGH, SOUTH SIDE	CAM- OF ROOT, 4″ DEEP IN SOIL	SOIL AT SIDE OF 2	CENTER OF STUMP, 1′ ABOVE GROUND	CAMBIUM 18′ HIGH, SOUTH SIDE	CAMBIUM OF ROOT, 4″ DEEP IN SOIL	IN SOIL AT SIDE OF 6	CAMBIUM 6″ HIGH, SOUTH SIDE	CENTER OF TREE, 1′ HIGH	CENTER OF TRUNK, 4′ HIGH	AIR TEMP.	BAROMETRIC PRESSURE	CONDITION OF WATER IN BARREL
	PARTLY CLEAR												
	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	°F.	in.	
2:00 P.M.	45.50	32.50	44.00	42.00	52.50	33.00	32.50	61.75	44.75	35.0	51.00	29.51	
3:00 "	47.00	32.75	48.00	44.25	50.25	32.00	32.00	57.25	46.50	37.5	46.50	29.52	
4:00 "	49.50	33.00	48.25	48.00	47.00	32.00	32.00	54.50	48.00	39.0	45.00	29.52	
7:00 "	41.00	32.75	35.50	49.50	39.00	32.00	32.00	42.00	47.50	44.5	37.5	29.57	
8:00 "	38.50	32.50	33.50	48.50	37.00	32.00	32.00	40.00	46.50	44.5	34.00	29.60	
10:45 "	33.00	32.00	32.75	42.00	31.50	32.00	32.00	33.50	39.00	42.5	31.75	29.66	Ice beginning to form
11:00 "	32.75	32.25	32.50	40.75	30.75	32.00	32.00	32.75	38.00	42.0	31.00	29.67	
11:15 "	31.75	31.50	32.00	39.50	29.50	32.00	31.50	32.00	37.00	41.5	31.00	29.67	
11:30 "	31.50	31.50	31.50	39.00	29.50	31.50	31.50	32.00	36.25	41.0	31.00	29.68	
11:45 "	31.50	31.50	31.50	38.50	29.50	31.50	31.50	32.00	36.00	40.5	31.00	29.68	Very thin ice; no water absorbed
APRIL 1, 1944	AIR TEMPERATURE CONTINUED TO DROP TO 29.0° F. AT 4:00 A.M., THEN TO 26.5° F. AT 6:00 A.M. CLEAR; NO WIND												
6:30 A.M.	29.25	32.75	32.00	32.00	32.00	32.50	32.50	31.50	32.00	32.0	28.50	29.82	1 liter of water absorbed
8:00 "	.....	.....	.....	.....	.....	.....	.....	.....	.....	30.5	31.00	29.86	

of water. The water level was carefully marked. This was done in order to determine whether that tree, under the same conditions of temperature and ample water supply as those in which the maple trees were placed, would also absorb water and ooze at least a small quantity of sap from a wound or tap-hole. During the period of observation, from then until April 12, the severed limbs did not absorb any measurable quantity of water from the barrel in which they were placed, either during the day or night. At no time did sap flow from severed branches.

Discussion

The results obtained by this series of experiments arouse some interesting speculations concerning the causes of the flow of maple sap. "Root pressure," or exudation pressure, was formerly considered one of the more important factors in the ascent of sap, but in recent years this view has been questioned by many. Its true significance, at least in the case of maples in early spring, appears to be that it provides a supply of water at the base of the trunk. Possibly the word "pressure" should not be used in this connection; "flow" might better serve to indicate what is taking place. Water from the soil enters the roots and passes along to the trunk of the tree. This cannot take place until the soil moisture is available or until the temperature of the tree at the frozen root-collar is high enough to permit water to pass up the trunk. Results with the severed trees indicate that the trunks and crowns of certain trees may be in condition for sap to flow for some time before the soil moisture is available.

Tree no. 2 (stripped trunk) started flowing as soon as it was tapped, which might be interpreted as not supporting the statement above. However, since there had been several days previous to this period when temperatures were well above freezing, it would have been possible for moisture to have been absorbed at that time. The control trees may also help to answer this objection. They did not start flowing simultaneously; in fact, one was several days later than the other. The only suggestion that can be made at present is that, for some reason as yet undetermined, the soil around one tree may have remained at a temperature below 32° F. longer that it did around the other. This could be due to varying density and duration of the shade given different areas by the tree tops.

The lifting power of transpiration has frequently been used to help explain sap flow, although it must be of small importance before the appearance of the leaves, and hence could not account for the flow of maple sap in the large quantities normally obtained. Tree no. 2 (stripped trunk) flowed freely as soon as it was tapped and continued to flow for 27 days, whenever the weather conditions were suitable. In the same way, tree no. 3 (stripped and severed) flowed at least 24 days. These are taken to indicate that not even the small amount of transpiration possible from buds and younger twigs is necessary to help lift sap through the trunk, since surfaces from which transpiration could have taken place were removed from tree no. 3.

To emphasize this further, tree no. 4 (cut in sections) from which sap flowed about as freely as from any of the other trees, could have had almost no transpiration whatever, as the top of each section was sealed with grafting wax.

It is improbable that the contraction of air in the xylem brought about by the change in temperature produces much, if any, of the force that causes sap to rise in maples. The quantities of sap involved are much too large. One might assume that capillarity is the force responsible. When, however, one section was removed from the barrel early in the morning and set in an empty basin, 850 ml. of sap drained out of the base within the next four hours.

In the red maple, then, sap will move up the trunk in early spring without benefit of root pressure from below and without the lifting power of transpiration from above. Since it moved equally well in trees no. 5 (severed, stripped, and inverted) and no. 6 (inverted and horizontal eight-foot sections) it seems clear that the position of the trunk is of little importance.

Here it should be emphasized that, regardless of the treatment given the experimental trees, and regardless of the fact that water was available at all times, sap flow occurred intermittently rather than continuously. Actually, it flowed only on those days and under those conditions in which maple orchard operators have learned to expect it to flow; i.e., during days in which the temperature of the air rises above freezing, following nights in which it goes below freezing (**1**, **5**). Both conditions are necessary. Freezing temperature results in sap rising in the trunk. As soon thereafter as the tree warms up, sap flows from the spouts. In fact, as soon as any part of the tree warms up sap will flow from a wound in that part.

Measurements made by means of thermocouples demonstrate that the cambium temperature may rise considerably above freezing early in the forenoon; it may, indeed, go well above the temperature of the air, especially on the side of the tree exposed to the sun (tables II, III). It also rises far more rapidly than does the temperature deeper in the trunk. The inner wood remains frozen until late in the day, and its temperature is likely to continue to rise due to thermal conduction for some little time after that of the air has again fallen below the temperature of the cambium. The cambium temperature, however, follows that of the air fairly closely; the water present there will start to freeze while that deeper in the xylem is still free to flow.

The following observations were made on the manner in which the water disappeared on several different occasions from the barrels surrounding the bases of the severed trees. A thin layer of ice formed on the surface, after which air appeared beneath it, and the volume of this air space gradually increased as the temperature of the atmosphere dropped. This indicated that the tree was then absorbing water. Since, at the same time, the cambium temperatures in the upper part of the trunk were at or below the

freezing point, the flow must have been into unfrozen tissue surrounded by frozen and freezing tissue. If the temperature dropped slowly, a large quantity of water was absorbed; on the other hand, if it cooled rapidly, considerably less water was taken in. Therefore, the rate of cooling had a considerable influence on the quantity of water which the trees absorbed and consequently must have had a controlling effect on the amount of sap which flowed from the spouts later on.

Apparently, the following is what takes place. The water in the cambium and outer layers of the trunk freezes first, leaving a concentrated sugar solution in the cells. As it freezes, the warmer sap or water moves toward the ice crystals as they form, from the areas of normal sugar content in the inner layers of the xylem. This results in a higher concentration of sugar in the latter cells producing a strong osmotic pull the length of the trunk. The water, as it moves toward the outside of the trunk, is replaced from the lower parts of the tree. Thus the direction of flow is through the roots and up the trunk to the upper parts of the tree where it is freezing. As the air becomes colder the sap freezes deeper in the wood; this continues until all the sap is frozen, from the cambium into the xylem to the heartwood and from the top of the tree to the ground or a little below it. Such a condition usually occurs sometime during the night, the time depending on how cold it gets and the rate of temperature fall.

To test this theory it was necessary to determine the presence or absence of ice in the wood of the tree following a period of freezing. Blocks cut from freshly felled trees and from the trunks and stumps of trees felled the day before were split open and examined under the low-power binocular microscope. Ice was observed glistening in all elements of the wood, rays as well as pores. Crystals were found in many places. Some of these were large, up to 1/50th-inch long and about one-fifth as thick, in the shape of rods or cylinders. When touched with a knife blade they could be seen to melt, the water flow towards the blade, and disappear. These were observed on both tangential and radial sections of the wood. On transverse sections, ice could not be seen; probably because it melted during the process of smoothing the wood. The conclusion is that these larger crystals were formed within the pores, rather than in intercellular spaces, none of which are evident in maple wood. It would seem that such a freezing process throughout the tissues might result in a decided expansion of the entire trunk, but the writers have had no opportunity to determine this. The only reference available is by MacDougal (7) who reports that daily variations in the diameter of a sugar maple in the New York Botanical Garden, as shown by a dendrograph, were most marked in March and April. The statement, however, is not clear and no data are presented.

The possibility was suggested that super-cooling prevented freezing and that ice crystals were not formed until the blocks were removed from the tree and split. To determine whether or not this occurred a heavy axe was used to jar the trunk at a distance of six inches from a thermocouple im-

bedded in it. This was done when that thermocouple registered four different temperatures (27°, 25°, 20° and 15° F.) on the potentiometer. There was no rise in temperature following the jar at any of those temperatures, a condition that should normally occur when heat of formation of ice is produced on freezing after super-cooling. This was taken as a positive indication that such super-cooling had not taken place and that any ice crystals observed had been produced previous to the time the section was removed from the tree.

This phenomenon of ice formation within the wood is not confined to maple or to other species exhibiting a copious flow of sap in the spring. Apple wood shows much the same condition. A six-inch block was removed from a limb three inches in diameter early in the morning of April 4, following a night when the temperature of the air had dropped to 17.0° F. Microscopic examination of the split block revealed very small ice crystals which were distributed throughout the xylem.

As the sun strikes the upper part of the maple tree, the crystals in the cambium and outer wood melt rapidly and the sap runs out at any available opening. JONES (5) has shown that sap flows first from the south side of trees warmed by the sun and from the outer inch of wood, although more sap is derived from the deeper layers which start flowing somewhat later. JONES and ORTON (6) found that sap moves through the wood lengthwise of the grain at a rate of from 2 inches to 4.8 inches a minute, in either direction. As the air temperature rises, more of the wood warms up and more ice melts. For a time this results in an increased rate of sap flow; later it decreases and finally stops. The exact reason why sap flow stops in the evening is not yet clear.

Summary and conclusions

1. Red maple trunks with both roots and crown removed produced sap of normal quality regardless of the position of the trunk, provided a supply of water was available.

2. Water moved into the trunks of severed trees only at the time during which air temperatures dropped below 32° F. This continued until the interiors of the trees were frozen. Ice was formed at this time in all tissues of the tree but was more plentiful in the outer xylem.

3. Sap started flowing from the spout as soon as the ice in that portion of the tree was melted. When water was available sap flow from the severed trees followed the same daily pattern as that from control trees, during the period of observation.

4. The flow of maple sap in early spring is not dependent on root pressure or on the lifting power of transpiration. It is probably brought about by the combined action of a number of factors, the most important of which is the daily change in temperature of the air.

5. Freezing temperature causes the formation of ice crystals in the phloem and outer layers of the xylem. Water from the inner layers of the

xylem is drawn toward the crystals as they form, thus setting up the tension which results in lifting sap from the roots to the upper parts of the tree. The process continues until it is stopped by freezing.

6. When released by thawing of the ice crystals, sap flows through the tissues and out at any convenient opening.

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EFFECTS OF POTASSIUM ON CHLOROPHYLL, ACIDITY, ASCORBIC ACID, AND CARBOHYDRATES OF *ANANAS COMOSUS* (L.) MERR.[1]

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(WITH THREE FIGURES)

Introduction

A study concerned with the effects of iron on chlorophyll, acidity, ascorbic acid, and carbohydrate fractions of *Ananas comosus* was reported in a previous publication (**35**). Also, the effects of different amounts of potassium on growth and ash constituents of the same plants were reported in a former paper of this series (**36**). This study is concerned with the effects of potassium on chlorophyll, acidity, ascorbic acid, and different carbohydrate fractions of the same plants.

The literature pertaining to the effects of potassium on chlorophyll emphasizes the fact that potassium deficiency may cause chlorophyll breakdown with the subsequent development of brown spots and leaf necrosis comparable to those observed in *A. comosus*. Wall (**46**) has recognized in tomatoes two types of potassium deficiency symptoms: the first stage marked by a stunted, hard, yellow plant was associated with a high carbohydrate content; and the second, in which the carbohydrate content had greatly diminished, the plant began to grow, turned green, became soft, and at the same time the lower leaves commenced to die progressively up the stem. Richards and Templeman (**31**) claim that symptoms of potassium deficiency in barley may either be a light yellow color of leaves associated with succulence and rapid death of the leaves or a dark green associated with white or brown spots on the leaves. According to Rohde (**32**), leaves of plants adequately supplied with potassium are yellowish green, while those deficient in potassium are deep green and contain more chlorophyll. No yellow color was observed in the leaves of *A. comosus* supplied with the low amounts of potassium indicated in this study, but instead a deep green color with a few brown spots and leaf tip necrosis was extensive in old leaves.

Our information pertaining to the effects of potassium on ascorbic acid is meager. However, ascorbic acid and total carboxylic acid reserves were closely associated with the chlorophyllose tissues of the plant (**35, 37, 39**). Comparable relationships between ascorbic acid and chlorophyllose tissues were shown by Giroud (**6, 7, 8**) for other plants. Ascorbic acid, according to Guha and Ghosh (**11**), is synthesized in *Phaseolus mungo* from mannose by an enzyme at pH 5.8 but not at 7.4. Ascorbic acid in the fruit of *A. sativus*, a synonym of *A. comosus* (L.) Merr., described as a reducing factor first by Szent-Györgyi (**42**), was reported to occur in fair concentrations

[1] Published with the approval of the Acting Director as Technical Paper no. 161 of the Pineapple Research Institute, University of Hawaii.

corresponding to that in the turnip. STOTZ *et al.* (**41**) consider the function of ascorbic acid in plant tissues comparable to that of cytochrome in animal tissues because both substances are rapidly reduced and oxidized by physiological respiratory systems. SZENT-GYÖRGYI (**43**) observed that tissues of cabbage leaves containing ascorbic acid were able to remove oxygen from the atmophere by means of the enzyme, ascorbic acid oxidase (hexoxidase), whereas those lacking ascorbic acid were unable to do so. His explanation was that "ascorbic acid plays an important part in the respiration of plants by connecting, as hydrogen carrier, the system in which the molecular oxygen enters into reaction." JAMES *et al.* (**21**) have observed that in the presence of ascorbic acid and atmospheric oxygen barley plant sap oxidized lactic acid to pyruvic acid.

The information pertaining to the effects of potassium on carbohydrate enzymes and carbohydrates is relatively extensive but not in perfect agreement on all points. VULQUIN and LISBONNE (**45**) state that diastase *in vitro* completely separated from neutral salts becomes inactive and resumes its activity only upon the addition of potassium and other metals. The viewpoint of KENDALL and SHERMAN (**23**) is that diastase accelerates the condensing as well as the hydrolytic reaction in carbohydrates. The studies of MASKELL (**25**) and JAMES (**19**) suggest that both condensing and hydrolytic reactions which may be designated as hexose-starch and starch-hexose reactions are dependent upon temperature, concentration of hexose, and concentration of enzyme. Fluctuations in the amount of starch in the leaves are not reflected in the velocity of starch-sugar reaction because starch is only slightly soluble and constitutes, therefore, a saturated solution. Any effect of potassium upon the rates of the reaction should be through the concentration of hexose or enzyme—a reduced hexose concentration during a period of darkness might lead to a faster disappearance of starch than during a period of light. Translocation of sugars is from high to low concentrations in the opinion of MASON and MASKELL (**26**). Diastase activity was increased by potassium in potato leaves (**19**), in bean leaves (**18**), and in Lemna (**48**) but was decreased in sugar cane (**12**) and sugar beets (**5**). HOAGLAND (**17**) claims that the activity of the various diastases may be modified in one direction or the other by a deficiency of potassium.

Plants supplied with small amounts of potassium contained in their leaves higher concentrations of reducing sugars than those with high potassium (**12, 22, 27, 28, 29, 42**). In totally potassium-deficient plants the amounts of reducing sugars were low (**9, 12, 16**) and according to GREGORY *et al.* (**9, 10**) they were due to low assimilation rate, high respiration, high protein synthesis, and excessive meristematic activity. High reducing sugar values in low-potassium plants are usually accompanied by low values in disaccharides (**12**) and polysaccharides, but the findings of WATSON (**47**) and RICHARDS (**30**) and RICHARDS and TEMPLEMAN (**31**) are in disagreement with HARTT (**12**) and those of the authors as reported below. Correlation between amounts of starch in the leaves and potassium supplied to the roots

was obtained in *Pisum sativum* by DAY and COMBONI (**4**) and JAMES (**19**), although starch accumulation in leaves is transient according to DAVIS as cited by HOAGLAND (**18**). Also, high starch in the stem correlated with a high potassium supply to the roots as shown in this and other studies (**22, 25, 27, 28, 29**). The low starch values obtained by WHITE (**48**) in the totally potassium-deficient cultures of Lemna may fall in the category characterized by GREGORY as due to a low assimilation rate, high respiration, high protein synthesis, and excessive meristematic activity. RUSSELL (**33**) claims correlation between the amounts of potassium supplied to roots and of fructosan polysaccharides in barley. According to HASTINGS (**13**), quoted by BARRON (**1**), the synthesis of glycogen in rat liver is increased fourfold if the slices are suspended in a solution containing potassium and magnesium at concentrations found in the intracellular fluid in the presence of glucose.

Methods

Techniques for the culture and sectioning of the plants and methods for the chemical analysis of the tissues and for the statistical treatment of the data are the same as those reported in previous publications (**35, 36, 37**).

Synoptical expressions replacing certain often repeated appellations in the text have been introduced for designating the different series. Thus the nitrate-nitrogen series is designated by N-n and the ammonium-nitrogen by A-n.

Results

DRY MATTER

Dry matter values, reported in table I as milligrams per gram of fresh weight, were higher in the leaves of the Low-K than High-K cultures in both nitrogen series, whereas in the stem similar values were higher in the High-K than Low-K cultures. The data in figure 2 and in table III show that the differences in dry matter between leaves and stem had resulted from a greater sugar content in the leaves of the Low-K cultures and a higher starch content in the stem of the High-K cultures. Total dry matter values per plant, reported in table VII, were higher in the High-K than Low-K cultures. Hemicelluloses, or celluloses plus lignin, although important items in dry matter, varied less with respect to amounts of potassium in the cultures than either sugars or starches.

TITRABLE ACIDITY

Titrable acidity, reported as milligrams of citric acid per gram of fresh tissue in table I and depicted in figure 1, was higher in the High-K than in the Low-K cultures. It was mostly restricted to the chlorophyllose sections of the leaves; the non-chlorophyllose sections of the leaves, or the stem and roots contain very low values as reported, also, in previous studies (**35**).

It is possible that the accumulation of greater amounts of organic acids in the Low-K cultures of the N-n than A-n series might have been favored by the higher calcium content of the plants, in accordance with the views of

various investigators (**3, 36, 38, 40**). However, later unpublished data suggest that diurnal changes and growth rates are concerned more directly with tissue acidity than other factors.

Ascorbic acid

Ascorbic acid values, reported in table II, were, with a few exceptions, higher for the Low-K than High-K cultures in the N-n series, but in the A-n

TABLE I

EFFECTS OF 205 VS. 4 MG. OF POTASSIUM PER LITER OF NUTRIENT SOLUTION SUPPLIED WITH EQUAL AMOUNTS OF NITRATE- OR AMMONIUM-NITROGEN ON THE DRY MATTER* AND TITRABLE ACIDITY, REPORTED AS CITRIC ACID† IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR.

PLANT SECTIONS	NITRATE-N				AMMONIUM-N			
	HIGH K		LOW K		HIGH K		LOW K	
	CITRIC ACID	DRY MATTER	CITRIC ACID	DRY MATTER	CITRIC ACID	DRY MATTER	CITRIC ACID	DRY MATTER
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves:								
Old (B) 1+2 (base)	23.9	134	0.7	138	30.0	126	18.3	142
3	78.8	122	7.0	128	70.3	124	52.3	134
4	113.0	130	97.0	152	114.0	135	85.2	157
5 (tip)	102.0	150	100.0	160	113.5	164	79.0	170
Mature (C) 1 (base)	23.2	112	22.0	118	34.8	112	16.1	118
2	73.8	122	68.0	122	47.2	108	22.2	118
3	123.0	125	114.5	140	137.7	117	75.7	148
4	152.0	126	151.0	165	158.7	141	101.3	170
5 (tip)	161.0	155	140.0	179	172.3	173	102.3	178
Active (D) 1 (base)	19.8	96	33.0	97	30.0	87	16.2	99
2	43.2	118	43.6	117	51.4	113	21.8	119
3	146.6	118	139.0	138	112.4	120	70.7	144
4	208.5	144	159.0	157	228.5	140	113.6	169
5 (tip)	227.0	159	173.5	177	212.3	172	130.5	184
Young (E) 1 (base)	30.2	86	31.7	87	38.5	83	18.6	86
2	38.0	100	48.6	105	43.0	105	23.2	112
3	179.0	125	154.0	125	138.0	130	65.7	137
4+5 (tip)	255.0	151	188.0	163	186.5	169	104.3	165
Stem:								
Base	15.1	212	28.5	158	31.6	184	22.7	154
Middle	31.6	203	36.5	140	31.9	160	33.4	144
Apex	50.4	127	47.5	122	52.8	110	36.3	124
Roots	8.7	149	11.5	166	8.7	149	7.3	137

* Milligrams per gram of fresh tissue.
† Milligrams per gram of dry tissue.

series they were higher for the High-K than Low-K cultures. The significance of the reversal of ascorbic acid values with respect to amounts of potassium and different kinds of inorganic nitrogen is not obvious at this time and cannot be explained. Ascorbic acid concentrations were very high in the chlorophyllose sections of the leaves in contrast to those in the non-chlorophyllose sections. However, such concentrations were not directly proportional to those of chlorophyll in different leaves of the same plant or in

similar leaves of different plants. Ascorbic acid and titrable acidity values correlated, being simultaneously either high or low in the same sections, although the values of the latter were consistently many times higher than those of the former.

Chlorophyll and carotenoids

Chlorophyll values varied in similar leaf sections of different cultures, as shown in table II. Chlorophyll differences between High-K and Low-K cultures in the N-n series in favor of the former cultures were statistically significant (odds 277 : 1). Also, similar differences in the A-n series but in favor of the Low-K cultures were statistically significant (odds 207 : 1). The

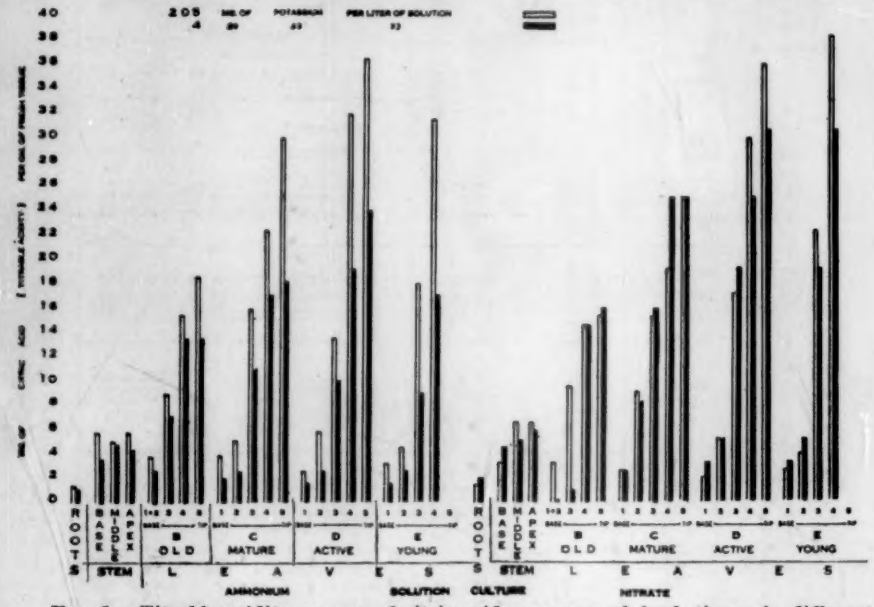

Fig. 1. Titrable acidity, as mg. of citric acid per gram of fresh tissue, in different sections of *A. comosus* grown in cultures supplied with 205 or 4 mg. of potassium per liter and with equal amounts of nitrate- or ammonium-nitrogen.

difference of the means of the chlorophyll content of the Low-K cultures between N-n and A-n series, in favor of the latter series, was statistically significant (odds 9999 : 1), but that of the High-K cultures lacked statistical significance.

The amounts of carotenoids and chlorophyll correlated in all leaf sections. The difference of the means between High-K and Low-K cultures in the N-n series, in favor of the High-K cultures (odds 64 : 1) was statistically significant; but in the A-n series, it was in favor of the Low-K cultures (odds 2500 : 1). A similar difference of the carotenoids of the Low-K cultures between N-n and A-n series, in favor of the A-n series (odds 9999 : 1) was statistically significant, but that of the High-K cultures lacked statistical significance (odds 3 : 1).

TABLE II

EFFECTS OF 205 VS. 4 MG. OF POTASSIUM PER LITER OF NUTRIENT SOLUTION SUPPLIED WITH EQUAL AMOUNTS OF NITRATE- OR AMMONIUM-NITROGEN ON CHLOROPHYLL, CAROTENOIDS, AND ASCORBIC ACID (MG. PER GRAM OF DRY TISSUE) IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* (L.) MERR.

PLANT SECTIONS	NITRATE-N						AMMONIUM-N					
	HIGH-K			LOW-K			HIGH-K			LOW-K		
	CHLOROPHYLL	CAROTENOID	ASCORBIC ACID	CHLOROPHYLL	CAROTENOID	ASCORBIC ACID	CHLOROPHYLL	CAROTENOID	ASCORBIC ACID	CHLOROPHYLL	CAROTENOID	ASCORBIC ACID
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves:												
Old (B) 1+2 (base)	1.61	0.187	0.2	1.49	0.174	0.2	1.51	0.151	0.3	1.36	0.169	0.2
3	4.90	0.753	2.7	3.10	0.446	5.9	4.15	0.540	2.7	4.40	0.641	1.9
4	4.57	0.761	8.5	4.17	0.618	15.7	5.19	0.800	18.3	4.77	0.704	6.7
5 (tip)	4.57	0.734	10.0	3.65	0.562	14.1	4.25	0.740	18.1	5.76	0.820	14.9
Mature (C) 1 (base)	.....	.....	0.1	.....	.....	0.2	.....	.....	0.1	.....	.....	0.3
2	1.63	0.188	1.1	1.94	0.213	0.3	2.06	0.250	0.6	2.13	0.245	0.5
3	4.21	0.608	7.4	3.67	0.550	11.0	3.70	0.488	9.9	5.16	0.682	3.8
4	3.90*	0.648	12.3	3.70	0.563	22.7	4.43	0.690	26.5	5.32	0.777	10.2
5 (tip)	4.71	0.807	11.3	3.71	0.537	13.8	5.49	0.787	31.3	5.38	0.878	13.2
Active (D) 1 (base)	.....	.....	0.1	.....	.....	0.2	.....	.....	0.5	.....	.....	0.3
2	1.61	0.161	0.1	1.22	0.137	0.2	1.92	0.097	0.4	1.45	0.151	0.3
3	3.63	0.475	0.9	3.59	0.428	2.5	3.72	0.433	1.6	4.77	0.610	0.2
4	4.55	0.656	30.6	3.52	0.705	25.9	4.12	0.614	41.6	4.73	0.752	10.6
5 (tip)	4.28	0.598	38.8	3.83	0.668	42.1	4.57	0.755	57.3	5.25	0.887	36.4
Young (E) 1 (base)	.....	.....	0.1	.....	.....	0.2	.....	.....	0.1	.....	.....	0.4
2	1.32	0.110	0.1	1.58	0.143	0.2	1.49	0.143	0.1	1.83	0.205	0.3
3	2.73	0.288	0.2	2.58	0.296	0.2	2.83	0.331	0.5	3.29	0.496	0.2
4+5 (tip)	3.81	0.570	12.9	3.58	0.490	20.6	2.71	0.415	25.3	3.94	0.643	8.3

* Doubtful.

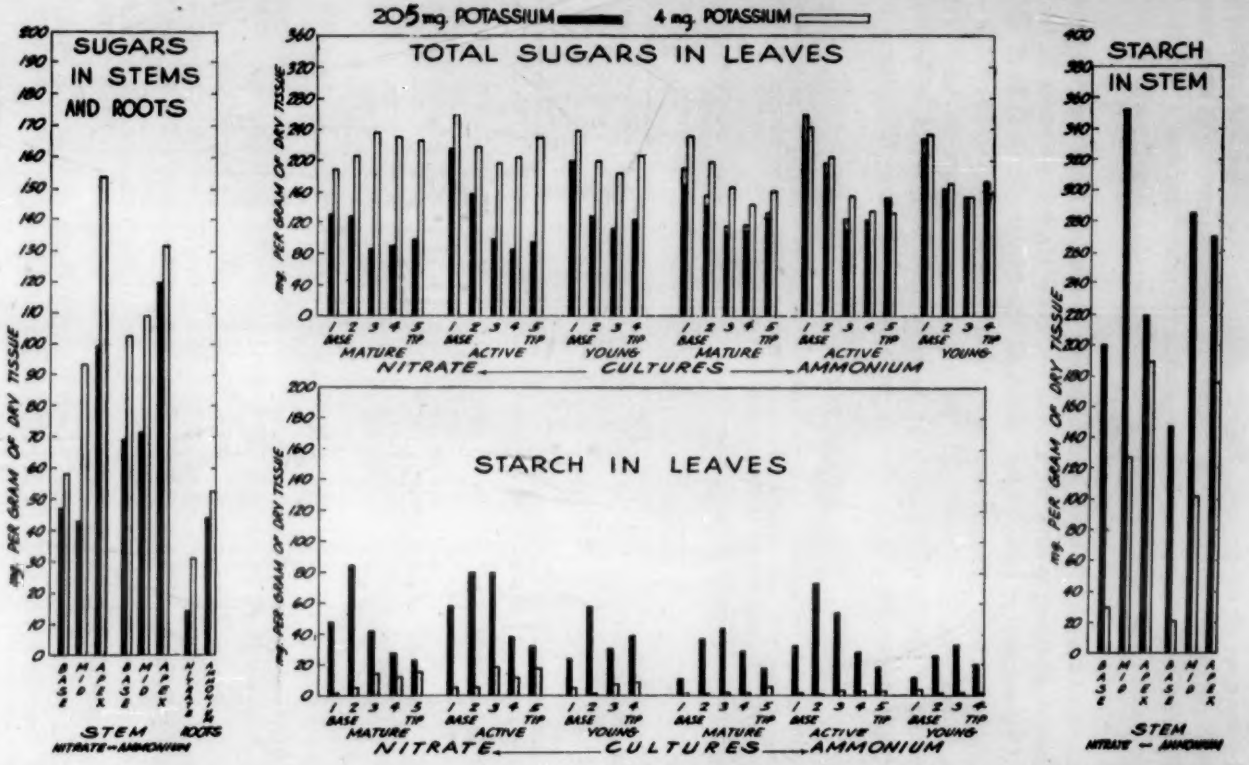

FIG. 2. Total sugars or starch, as mg. per gram of dry tissue, in different sections of *A. comosus* grown in cultures supplied with 305 or 4 mg. of potassium per liter and with equal amounts of nitrate- or ammonium-nitrogen.

The fact that chlorophyll and carotenoid values were higher in the High-K cultures of the N-n series and in the Low-K cultures of the A-n series contradicts any possible effects of potassium on the chlorophyll and carotenoid content of the leaves. Other factors possibly associated with the relations of potassium to nitrate or ammonium assimilation might have been responsible for such differences as indicated in the above results.

Sugars

Total sugar values, depicted in figure 2, as per mil of fresh weight, were, with a few exceptions, greater in the Low-K than High-K cultures in both series. Comparison of total sugar values of the leaves of the High-K cultures shows that they were greater in the A-n than N-n series, but those of the Low-K cultures were reversed. Reducing sugar values, in table III, were greater for the Low-K than High-K cultures in both series, indicating that potassium supplied in insufficient amounts to the Low-K cultures re-

TABLE III

EFFECTS OF 205 VS. 4 MG. OF POTASSIUM PER LITER OF NUTRIENT SOLUTION SUPPLIED WITH EQUAL AMOUNTS OF NITROGEN EITHER AS NITRATE OR AMMONIUM ON THE AMOUNTS OF REDUCING SUGARS* AND SUCROSE*

Plant sections	N-n series				A-n series			
	High K		Low K		High K		Low K	
	Reducing sugars	Sucrose	Reducing sugars	Sucrose	Reducing sugars	Sucrose	Reducing sugars	Sucrose
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves:								
Old (B) 1+2 (base)	87.5	23.2	144.0	21.0	94.0	30.0	109.5	30.0
3	98.7	37.7	184.0	31.0	117.5	35.5	142.0	33.0
4	94.4	33.9	195.0	31.0	100.0	40.0	142.0	35.0
5 (tip)	86.0	38.0	181.0	33.0	111.0	44.0	156.0	44.0
Mature (C) 1 (base)	107.0	23.0	164.0	23.7	161.0	30.0	203.0	30.0
2	96.0	32.0	189.0	16.0	126.4	28.6	175.0	25.0
3	51.2	35.3	201.0	35.0	79.0	37.0	130.0	37.0
4	48.6	41.4	192.5	37.5	73.3	44.7	110.0	35.0
5 (tip)	55.0	42.0	182.5	44.5	89.0	45.0	117.0	44.0
Active (D) 1 (base)	195.0	20.0	238.0	21.0	239.3	20.7	225.0	19.0
2	136.0	22.0	199.0	18.0	176.0	22.0	187.0	19.0
3	70.0	29.0	171.3	24.7	89.3	34.2	119.0	36.0
4	45.8	40.2	174.4	30.6	79.1	44.4	98.5	36.0
5 (tip)	56.5	38.5	190.0	38.0	108.0	44.0	94.2	37.8
Young (E) 1 (base)	170.0	32.0	209.0	30.0	197.5	30.5	205.0	29.0
2	110.0	19.0	186.0	12.0	143.0	20.0	149.0	21.0
3	88.0	26.0	163.0	20.0	126.0	28.0	132.0	22.0
4+5 (tip)	83.0	42.0	177.0	30.0	130.5	42.5	130.0	27.0
Stem:								
Base	19.6	27.4	40.3	17.7	46.1	22.9	80.0	22.0
Middle	12.8	30.2	63.0	30.0	46.4	25.0	84.0	25.0
Apex	56.3	41.7	105.7	47.3	76.3	43.7	84.0	47.6
Roots	2.7	12.0	8.4	22.3	23.6	20.0	28.1	23.8

* Milligrams per gram of dry tissue.

sulted in accumulations of variable magnitudes of total and reducing sugars in both leaves and stem. Comparison of the difference of reducing sugars as percentage of total sugars between Low-K and High-K cultures (table IV) shows that such differences increased progressively in the N-n series from the basal (no. 1) to the terminal (no. 5) sections of the leaves and from the apical to the basal sections of the stem. In the A-n series similar differences between High-K and Low-K cultures were not as great as in the N-n series indicating that the rate of conversion of reducing sugars to more complex carbohydrate compounds had possibly been retarded either by the primary products of ammonium assimilation or by Cl ions present in the solution cultures of High-K cultures in the A-n series.

TABLE IV

DIFFERENCE BETWEEN LOW-K AND HIGH-K CULTURES IN REDUCING SUGARS AS PERCENTAGE OF TOTAL $\frac{\text{R.S. (LOW-K)} \times 100}{\text{T.S. (LOW-K)}} - \frac{\text{R.S. (HIGH-K)} \times 100}{\text{T.S. (HIGH-K)}}$ IN DIFFERENT PLANT SECTIONS*

Series	Leaves						Stem	
	Sections	Old	Mature	Active	Young	Average	Sections	
		%	%	%	%	%		%
N-n	1	8.3	4.9	1.2	3.3	4.4	Base	27.5
	2	.....	17.1	5.9	8.8	10.6	Middle	37.8
	3	13.1	26.1	16.8	12.0	17.0	Apex	11.6
	4	13.0	29.8	31.6	19.1	23.4	.....	.....
	5	15.3	23.7	23.8	.....	20.9	Roots	7.8
A-n	1	4.2	2.9	0.0	0.9	2.0	Base	11.8
	2	.....	7.2	1.8	0.0	3.0	Middle	12.0
	3	4.2	9.8	4.3	4.0	5.6	Apex	0.7
	4	8.5	13.7	9.2	7.5	9.7	.....	.....
	5	6.4	6.2	0.3	.....	3.25	.....	.....

* R.S. = Reducing Sugars; T.S. = Total Sugars.

Sucrose values (table III) were, with a few minor exceptions, greater in the High-K than in the Low-K cultures. In table V, mean sucrose values as percentage of total sugars in the N-n series were 27.1 and 53.4 per cent. respectively, for the leaves and stem of the High-K cultures; those of the Low-K cultures were 12.9 per cent. for the leaves and 31.0 per cent. for the stem. Similar values in the A-n series were 23.5 per cent. for the leaves and 35.0 per cent. for the stem of the High-K cultures and for the Low-K cultures leaf values were 18.45 and stem values 27.0 per cent. They indicate that in the N-n series sucrose synthesis from reducing sugars was 2.10 and 1.72 times greater for the leaves and stem, respectively, in the High-K than Low-K cultures. A similar synthesis in the A-n series was 1.27 and 1.30 times greater for the leaves and stem, respectively, in the High-K than Low-K cultures. Considering the chlorophyllose sections (nos. 3–5) alone in the N-n series, sucrose values were from 2.0 to 3.2 times greater in the High-K than in the Low-K cultures. These values, being much higher for the chlo-

rophyllose sections than for the entire leaf, suggest the possibility of a greater rate of sucrose synthesis from reducing sugars in the chlorophyllose than in the non-chlorophyllose sections. On the basis of this assumption accumulations in the basal leaf sections or in the stem may indicate sucrose translocation but not synthesis in situ. Comparison in table III of the

TABLE V

SUCROSE AS PERCENTAGE OF TOTAL SUGARS AND STARCH AS PERCENTAGE OF TOTAL AVAILABLE CARBOHYDRATES (SUGARS PLUS STARCHES) IN DIFFERENT SECTIONS OF ONE-YEAR-OLD *Ananas comosus* GROWN IN SOLUTION CULTURES CONTAINING EQUAL AMOUNTS OF NITROGEN FROM NITRATE OR AMMONIUM SALTS BUT EITHER 205 OR 4 MG. OF POTASSIUM PER LITER OF SOLUTION

PLANT SECTIONS	SUCROSE				STARCH			
	N-N		A-N		N-N		A-N	
	HIGH-K	LOW-K	HIGH-K	LOW-K	HIGH-K	LOW-K	HIGH-K	LOW-K
	%	%	%	%	%	%	%	%
Leaves:								
Old (B) 1+2 (base)	19.8	12.7	24.2	21.6	13.1	1.5	6.9	0.4
3	27.6	14.8	23.2	18.9	16.6	5.0	11.7	1.9
4	26.4	13.7	28.6	19.8	9.3	4.0	11.0	1.6
5 (tip)	14.5	15.4	28.3	22.0	7.2	5.0	5.7	1.6
Mature (C) 1 (base)	17.7	12.6	15.7	12.9	25.0	0.8	5.2	0.3
2	25.0	7.8	18.4	12.5	37.0	2.4	17.8	0.4
3	40.8	14.8	31.9	22.2	30.5	5.2	26.0	1.9
4	46.0	16.3	37.9	24.0	21.7	4.3	18.1	1.5
5 (tip)	43.3	19.6	33.6	27.3	17.3	5.9	10.8	3.4
Active (D) 1 (base)	9.3	8.1	8.0	7.8	19.7	1.8	10.3	0.7
2	13.9	8.3	11.1	9.2	31.3	2.1	25.0	0.3
3	29.3	12.6	27.7	23.1	41.8	7.7	28.6	2.3
4	46.8	14.9	35.9	26.7	28.1	5.1	16.5	1.6
5 (tip)	40.6	16.7	29.0	27.9	23.3	6.1	9.7	1.8
Young (E) 1 (base)	15.8	12.5	13.4	12.4	9.4	1.7	4.5	1.5
2	14.7	6.1	12.3	12.3	35.2	0.5	13.1	0.5
3	22.8	10.9	18.2	14.3	19.1	3.4	16.2	0.9
4 (tip)	33.6	14.5	24.5	17.2	21.9	3.4	9.4	1.0
Stem:								
Base	57.5	30.8	33.4	21.6	79.4	30.6	65.8	16.0
Middle	70.0	32.3	35.1	23.0	88.3	55.2	78.4	45.8
Apex	32.2	30.9	36.4	36.3	66.8	76.4	67.0	83.0
Roots	82.0	73.0	46.0	45.8	28.8	16.2	.....	.....

effects of different kinds of inorganic nitrogen on the polymerization of reducing sugars show that such effects were greater in the High-K than Low-K cultures and were more effective in the N-n than in the A-n series. Ammonium ions supplied to the A-n series tended to retard the rate of conversion of reducing sugars to starch whereas nitrate-N, regardless of amounts supplied, interfered little or none with such processes. Sucrose, reported in table V as percentage of total sugars, was higher in the High-K than Low-K cultures in both nitrogen type series, indicating that synthesis of this disaccharide was promoted more by high than low amounts of potassium.

Starch

Starch, more than any other organic constituent of the tissues, was affected by the amounts of potassium in the solution cultures. Being a reserve carbohydrate it accumulated in the stem from 10 to 20 times more than in the leaves, as depicted in figure 2.

Starch depositions in the leaves do not present the same gradients as inorganic salts; the former are restricted to sections irrespective of polar orientation whereas the latter, influenced by the transpiration stream, tend to increase gradually from the basal to the terminal sections. Starch depositions in the active (D) leaves of High-K cultures were greatest in the tissues of relatively low photosynthetic and growth activity, as in the transitional (no. 2) and low chlorophyllose (no. 3) sections; in the Low-K cultures, the low chlorophyllose (no. 3) contained more starch than the transitional (no. 2) or other sections. In other leaves, with different rates of growth and metabolic activity, starch accumulations were also found in other leaf sections besides the transitional (no. 2). The above comparisons, indicating that the starch content of the transitional (no. 2) section was affected more by the potassium content in the cultures than other sections, suggest its possible use as an index of plant responses to potassium treatments. The information so far available, indicating that ammonium ions supplied to solution cultures decrease more than nitrate ions the rate of starch synthesis and accumulation in such sections, suggests that interpretations of data on starch findings should consider any inter-relationships as might exist between K, NH_4, or NO_3 ions.

The starch content of different stem sections varied; it was higher in the medial than apical sections of the High-K cultures and lower in the medial than apical sections of the Low-K cultures. The basal stem sections in all cultures contained lower amounts of starch than others. Starch as percentage of total available carbohydrates (sugars plus starches), reported in table V, was from 3 to 30 times higher in the mature (C) and active (D) leaves of the High-K than Low-K cultures in the N-n series. Differences in stem starch values for the different sections between High-K and Low-K cultures (table VI) increased in favor of the former cultures as follows: in the apical, 35 per cent. ($16 + 54 \div 2 = 35$); in the medial, 179 per cent. ($177 + 180 \div 2 = 179$); and in the basal sections, 600 per cent.

Starch deposition differences in the Low-K cultures between apical and medial stem sections, in favor of the former sections, were 49 and 72 per cent., respectively, for the N-n and A-n series; those between apical and basal sections, not shown in table VI, also in favor of the former, were 552 and 773 per cent., respectively; those between medial and basal sections, in favor of the former, were 340 and 385 per cent., respectively. Similar differences in the High-K cultures between apical and medial stem sections, in favor of the latter, were 61 and 6 per cent., respectively, for the N-n and A-n cultures; those between the apical and basal sections, not shown in table VI, also in favor of the former, were 9.6 and 83.7 per cent., respectively; and those be-

tween the medial and basal sections, in favor of the former, were 76 and 94 per cent., respectively. Hence the gradients of starch deposition from the apical to the basal sections decreased 552.0 and 9.5 per cent., respectively, for the Low-K and High-K cultures, which indicates that the rate of starch deposition was 58.1; i.e., (552.0 ÷ 9.5) times lower in the Low-K than High-K cultures. In the A-n series similar gradients, also decreasing from the apical to the basal sections, were lower, 733.0 and 83.7 per cent., respectively, for the Low-K and High-K cultures, indicating that the rate of starch deposition was 8.76; i.e., (733.0 ÷ 83.7) times lower in the Low-K than High-K cultures.

TABLE VI

COMPARATIVE ANALYSES OF THE PERCENTAGE DIFFERENCES IN STARCH IN STEM SECTIONS BETWEEN HIGH- AND LOW-POTASSIUM CULTURES, BETWEEN NITRATE- AND AMMONIUM-NITROGEN SERIES, OR BETWEEN MEDIAL- AND APICAL- OR BASAL-SECTIONS

ITEM NO.	COMPARISON	STEM SECTIONS	CULTURES	SERIES	DIFFERENCE MG./GM.	PERCENTAGE
1	H-K vs. L-K	Apical	H-K,* L-K†	N-n	219–189 = 30	16
2	H-K vs. L-K	Medial	H-K, L-K	N-n	352–127 = 225	177
3	H-K vs. L-K	Basal	H-K, L-K	N-n	200– 29 = 171	600
4	H-K vs. L-K	Apical	H-K, L-K	A-n	270–175 = 95	54
5	H-K vs. L-K	Medial	H-K, L-K	A-n	285–102 = 183	180
6	H-K vs. L-K	Basal	H-K, L-K	A-n	147– 21 = 126	600
7	N-n vs. A-n	Apical	H-K	N-n, A-n	219–270 = 51	– 23
8	N-n vs. A-n	Medial	H-K	N-n, A-n	352–285 = 67	24
9	N-n vs. A-n	Basal	H-K	N-n, A-n	200–147 = 53	26
10	N-n vs. A-n	Apical	L-K	N-n, A-n	189–175 = 14	8
11	N-n vs. A-n	Medial	L-K	N-n, A-n	127–102 = 25	25
12	N-n vs. A-n	Basal	L-K	N-n, A-n	28– 21 = 7	33
13	Med. vs. Ap.	Med.-Ap.	H-K	N-n	352–219 = 133	61
14	Med. vs. Bas.	Med.-Bas.	H-K	N-n	352–200 = 152	76
15	Med. vs. Ap.	Med.-Ap.	L-K	N-n	127–189 = –62	– 49
16	Med. vs. Bas.	Med.-Bas.	L-K	N-n	127– 29 = 98	340
17	Med. vs. Ap.	Med.-Ap.	H-K	A-n	285–270 = 15	6
18	Med. vs. Bas.	Med.-Bas.	H-K	A-n	285–147 = 138	94
19	Med. vs. Ap.	Med.-Ap.	L-K	A-n	102–175 = –73	– 72
20	Med. vs. Bas.	Med.-Bas.	L-K	A-n	102– 21 = 81	385

* H-K = high-potassium cultures.
† L-K = low-potassium cultures.

Stem starch values reported as percentage of dry matter in table VII were 27.18 and 11.72, respectively, for the High-K and Low-K cultures in the N-n series while in the A-n series they were 23.45 and 8.8 per cent., respectively, for the High-K and Low-K cultures, indicating 2.32 and 2.66 times more starch in the High-K than Low-K cultures for the N-n and A-n series, respectively.

The comparisons of starch deposits in sections 2 and 3 of the leaves between High-K and Low-K cultures bear out approximately the same relations as those of the stem.

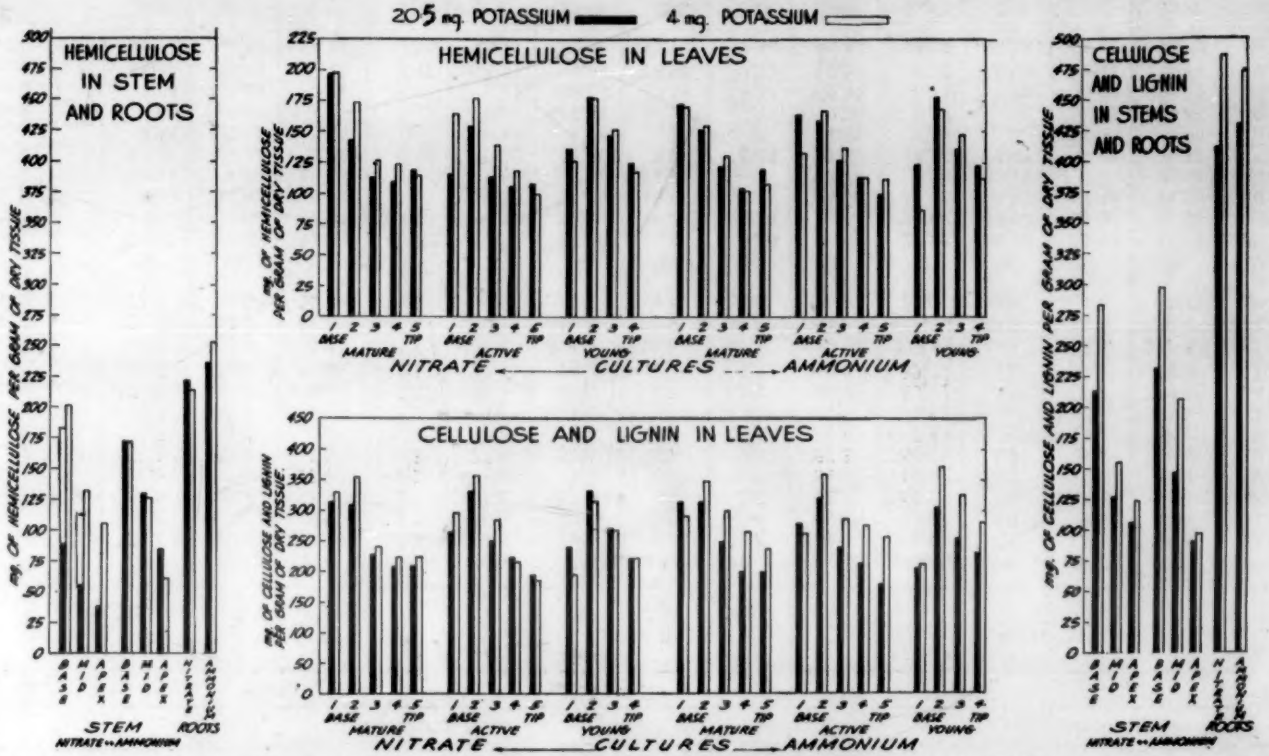

FIG. 3. Hemicelluloses or celluloses and lignin, as mg. per gram of dry tissue, in different sections of *A. comosus* grown in cultures supplied with 205 or 4 mg. of potassium per liter and with equal amounts of nitrate- or ammonium-nitrogen.

TABLE VII

TOTAL AMOUNTS IN GRAMS OF OBSERVED DRY MATTER (D.M.) AND RECOVERED DRY MATTER IN GRAMS OR PERCENTAGE, DETERMINED BY ANALYSIS AS CELLULOSE-LIGNIN, HEMICELLULOSE, SUGAR, STARCH, CITRIC ACID, ASCORBIC ACID, CHLOROPHYLLOSE PIGMENTS, ORGANIC NITROGEN (×6.25), AND ASH PER PLANT OF ONE-YEAR-OLD *Ananas comosus* GROWN IN SOLUTION CULTURES WITH 205 OR 4 MILLIGRAMS OF POTASSIUM PER LITER AND WITH EQUAL AMOUNTS OF NITROGEN EITHER AS NITRATE OF AMMONIUM

Substances	Nitrate series								Ammonium series							
	High-K				Low-K				High-K				Low-K			
	Plant	Leaves	Stem	Roots	Plant	Leaves	Stem	Roots	Plant	Leaves	Stem	Roots	Plant	Leaves	Stem	Roots
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Cellulose-lignin	129.3	100.7	8.3	20.3	104.5	79.8	4.4	20.3	123.0	102.6	7.8	12.6	99.5	75.9	3.6	20.0
Hemicellulose	66.6	52.7	6.9	10.4	56.1	42.6	3.2	10.3	68.7	55.2	6.5	7.0	45.8	32.9	2.2	10.7
Sugar	51.6	47.4	3.5	0.7	71.1	67.2	2.4	1.5	71.1	65.5	4.3	1.3	45.3	43.4	1.9	.....
Starch	32.9	17.0	15.8	0.1	6.2	3.4	2.8	+*	24.0	12.3	11.7	+	2.2	0.7	1.5	.....
Citric acid	57.1	54.9	1.9	0.3	37.9	36.4	0.9	0.6	57.3	55.1	1.9	0.3	20.3	19.8	0.5	.....
Ascorbic acid	4.7	4.7	.....	.....	3.4	3.4	.....	.....	7.6	7.6	.....	.....	2.1	2.1	.....	.....
Chloroph. pigments	1.5	1.5	.....	.....	1.0	1.0	.....	.....	1.5	1.5	.....	.....	1.1	1.1	.....	.....
Org.-N (×6.25)	36.9	31.9	3.8	1.2	31.9	26.2	2.6	3.1	40.0	35.0	3.1	1.9	38.8	34.4	2.5	1.9
Ash	59.6	52.0	5.5	2.1	18.7	14.3	3.1	1.3	45.8	39.7	4.8	1.3	14.2	11.6	1.4	1.2
Recovered D.M.	440.2	362.8	45.7	35.1	330.8	274.3	19.4	37.1	439.0	374.5	40.1	24.4	269.3	221.9	13.6	33.8
Observed D.M.	511.4	406.3	58.1	47.0	386.7	314.8	23.9	48.0	504.0	424.7	49.9	29.3	320.0	261.0	17.0	42.0
Not recovered D.M.	71.2	43.5	12.4	11.9	55.9	40.5	4.5	10.9	65.0	50.2	9.8	4.9	50.7	39.1	3.4	8.2
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Cellulose-lignin	25.25	26.35	14.28	43.25	27.08	25.40	18.40	42.30	24.42	24.20	15.63	43.00	31.10	29.08	21.05	47.65
Hemicellulose	13.04	13.00	11.88	22.15	14.08	13.55	13.38	21.70	13.64	13.00	13.03	23.90	14.30	12.62	12.94	25.50
Sugar	10.10	11.67	6.02	1.50	18.36	21.30	10.05	3.13	14.10	15.42	8.82	4.44	14.20	16.62	11.18	.....
Starch	6.44	4.19	27.18	0.20	1.60	1.08	11.72	+	3.77	2.90	23.45	+	0.69	0.27	8.80	.....
Citric acid	11.17	13.52	3.27	0.64	9.80	11.56	3.77	1.25	11.37	12.95	3.81	1.03	6.35	7.59	2.90	.....
Ascorbic acid	0.92	1.16	.....	.....	0.88	1.08	.....	.....	1.51	1.79	.....	.....	0.66	0.80	.....	.....
Chloroph. pigments	0.29	0.37	.....	.....	0.26	0.32	.....	.....	0.30	0.35	.....	.....	0.37	0.42	.....	.....
Org.-N (×6.25)	7.24	7.89	6.54	2.55	8.27	8.33	10.88	6.46	7.94	8.24	6.22	6.50	12.12	13.18	14.70	4.53
Ash	11.67	12.80	9.47	4.47	4.84	4.54	12.97	2.71	9.10	9.34	9.62	4.44	4.44	4.45	8.24	2.86
Recovered D.M.	86.12	90.95	78.64	74.76	85.17	86.86	81.17	77.55	86.15	88.19	80.58	83.31	84.23	85.03	79.81	80.54
Fresh weight (gm.)	3859	3210	335	314	2741	2280	173	288	3869	3330	342	197	2250	1820	120	310

* + = traces (not measurable).

Hemicelluloses

Hemicellulose values reported as milligrams per gram of dry tissue in figure 3 differed slightly between Low-K and High-K cultures. Differences in the hemicellulose content of the leaves between different cultures in the same series were small and insignificant. Thus the pattern for the hemicellulose distribution in different cultures did not conform in all respects to that for sugars depicted in figure 2. Certain small differences appearing in the data were possibly caused by the different sources of nitrogen rather than by the amounts of potassium in the cultures. Therefore, the physiological processes involved in hemicellulose deposition may differ from those of starch. Former studies (**35**) have also suggested that hemicelluloses in *A. comosus* do not represent reserves of readily available carbohydrates, as sugars or starch, but that they are allied more to structural substances and are comparable in this respect to celluloses and lignin.

The data in table VII, reporting hemicellulose as percentage of dry matter as well as total amounts per plant, show that the percentage values were approximately the same in both leaves and stem but in the roots they were almost twice as great as in the former organs. The percentage of hemicellulose content of the dry matter of the leaves, stem, and roots in the different cultures was relatively uniform for the specific organ regardless of great differences in the amounts of potassium and kinds of inorganic nitrogen between different cultures.

Cellulose-lignin

The combined weights of these compounds (fig. 3) were, with minor exceptions of certain sections, significantly greater for the Low-K than High-K cultures in the A-n series, but in the N-n series similar differences lacked statistical significance. The cellulose-lignin distribution was comparable to that of hemicelluloses in the different cultures. The data in table VII, reporting cellulose-lignin as percentage of dry matter as well as total amounts per plant, show that percentage cellulose-lignin values in the stem were approximately one-half as great as in the leaves and those in the leaves one-half as great as in the roots. The higher cellulose-lignin values in the leaves and roots than in the stem are directly related to the lignocellulose values of the fibrovascular system which occupies a much greater volume and area in the former than latter organs.

Discussion

Understanding of the physiological function of potassium in carbohydrate synthesis is beclouded by the lack of harmony in the results of different investigators. Hibbard and Grigsby (**16**) have summarized their studies with the statement that: "Any attempt to prove that any particular element aids directly or indirectly in the synthesis of carbohydrates or proteins, is instrumental in interrupting hydrolysis or condensation of starch, or affects the regulation of the translocation of inorganic or of elaborated foodstuffs will lead one now only into a cul-de sac." This statement, taking into con-

sideration the disharmonious conclusions of various workers, expresses without exaggeration the approximate state of our information on the physiological functions of potassium. SCHMALFUSS (34) remarks that potassium acts indirectly on proteins and carbohydrates; its primary function in the life processes of the plant must be sought in the colloidal activity of K-ions.

The absence of organic compounds containing potassium in plant tissues and the almost complete recovery of this element from the sap of plants in ionic state suggest that it plays an indirect rôle in most biological processes in the cell.

The data in table VII, reporting total amounts per plant of cellulose-lignin, hemicellulose, starch, citric acid, ascorbic acid, chlorophyllose pigments, organic-N, and ash as well as values of the same as percentage of dry matter, show certain inter-relationships which deserve further comment. The cellulose-lignin and hemicellulose fractions as percentage of dry or fresh matter were highest in the roots; the former being almost twice as great as the latter. Cellulose-lignin values as percentage of dry matter were almost half as great in the leaves as in the roots but twice as great as in the stem, but those of hemicellulose were approximately the same in both leaves and stem. Except for the differences mentioned in the cellulose-lignin and hemicellulose fractions which may be classified as organogenic, other differences resulting from cultural treatments were relatively absent. Therefore, it may be assumed that hemicelluloses and lignocellulose substances are components of structural units and undergo little or no change in metabolic processes requiring energy from carbohydrates. Sugar, starch, and organic acids, however, either carboxylic or amino acids, being intermediate products of metabolism and subject to further oxidation or reduction, vary greatly in different organs because of variations in the physiological function and metabolism of such organs. Differences in cultural treatments, as High-K vs. Low-K or NO_3 ions vs. NH_4 ions affecting the tempo or course of metabolic processes, change greatly the relative content of the tissues in sugar, starch, or organic acids but not in hemicellulose or ligno-cellulose substances.

Titrable acidity, in agreement with the findings of other investigators who have observed that tissue acidity may be increased by increasing the concentration of the nitrate salts of Na, K, or Ca, was higher in the High-K than Low-K cultures. However, it was suggested by certain unpublished studies that, owing to the great fluctuations in acidity between day and night samples, between samples collected during cloudy or sunny weather, or between plants of a different rate of growth and vigor, the differences between High-K and Low-K cultures might have been influenced, at least in part, by these climatic and physiological factors as well as the amounts of potassium in the cultures.

The data on ascorbic acid suggest that the amounts of potassium within the limits of this study did not influence the ascorbic content of the tissues. If mannose serves as the primary substance for the synthesis of ascorbic acid, according to GUHA and GHOSH (11), then information pertaining to the rôle

of potassium on mannose synthesis might have explained the behavior of the plants towards the ascorbic acid content of the tissues. The results on chlorophyll and carotenoids were inconsistent and indecisive on account of the more dominating rôle exerted by the different sources of nitrogen rather than by potassium.

In view of the transient nature of starch in the leaves, it is advisable to employ, for critical studies, other organs of the plant with food storage parenchymatous tissues (fruits, stem, tubers, etc.) where starch accumulations or depletions are exaggerated. The gradients of starch distribution in the stem of *A. comosus* as reported in table VI may be explained as follows:

The differences in the starch values of the stem between the High-K and Low-K cultures, being relatively small in the apical regions but becoming greater in the medial and basal sections, suggest that starch deposition under conditions of restricted starch synthesis, as in the Low-K cultures, was limited only to the tissues of the apical sections and that as these tissues advanced in age and their position was taken by new ones the amount of starch they received was progressively reduced. This condition was brought about either by a restricted sugar translocation or by a reversal in the ratio of synthesizing to hydrolyzing starch enzymes. The lower amounts of starch in the High-K cultures of the A-n than N-n series should be attributed to a lower rate of sugar polymerization in the former series. The higher amounts of starch in the medial than in the apical sections of the High-K cultures may indicate a greater differentiation in the relative amounts of storage tissue in the former sections on account of inherent differences in the chronological age and physiological maturity of the tissues. The lower starch content in the basal than in the medial stem sections of the High-K cultures should be attributed also to differences in the relative amounts of starch storage tissue which in the basal sections decreased on account of a greater hemicellulose and cellulose-lignin content. The higher starch content in the apical than the medial or basal stem sections of the Low-K cultures should be attributed to a gradually decreasing rate of conversion of sugar to starch and deposition of the latter from the apical to the basal sections. The changes in the rate of conversion of sugar to starch were induced, in the opinion of the authors, by enzymes operating differently under conditions of insufficient amounts of potassium.

LISSYZIN'S (**24**) interpretation of the mechanism of starch formation from sucrose follows the scheme: sucrose $\rightleftharpoons$ monose $\rightleftharpoons$ dextrins $\rightleftharpoons$ starch, which shows that no direct transformation of sucrose into starch takes place and also that hydrolysis of starch to sucrose proceeds through monose formation as the intermediate stage. Phosphorylation is apparently the key and lock in various phases of carbohydrate metabolism. BARRON (**1**), explaining the mechanism of carbohydrate breakdown and polymerization in plants as demonstrated by JAMES and HANES, says: "The existence of the first phase, carbohydrate $\rightarrow$ pyruvate has been shown by JAMES *et al.* (**19**)

in the metabolism of barley when pyruvic acid was formed by barley sap from glucose in the presence of adenylic acid and the necessity of phosphorylation for carbohydrate breakdown demonstrated by the ratio of O_2 formation to inorganic P disappearance. The importance of phosphorylation for carbohydrate metabolism by plants has been corroborated by HANES (**14, 15**) on the breakdown and synthesis of starch by phosphorylase, quite similar to CORI's phosphorylase, but isolated from potato and pea seeds." The rôle of potassium or of nitrogen either as nitrate or ammonium in phosphorylase activity has not been adequately studied to offer a concrete explanation of the findings. It may be assumed, however, on the basis of the data herein presented, that cultural treatments with nitrogen or potassium, affecting protoplasmic activity and, in turn, the course of enzymatic reactions, may accelerate or retard the mechanism of starch synthesis and its deposition in different organs.

Tissue hydration often associated with the physiological functions of potassium by various investigators shows little or insufficient evidence in its favor. Although the higher water content in the stem of the Low-K cultures might have interfered with the condensation of sugars and their conversion into starches, this argument does not apply to the leaves where high sugars were associated with a lower water content.

The data suggest that the better plant growth obtained with adequate than with deficient amounts of potassium may be caused by a better synthesis, polymerization, and assimilation of readily available carbohydrates and other vitally essential substances.

Summary

1. The amounts of chlorophyll and carotenoid pigments in the leaves were not affected by the amounts of potassium in the cultures. Certain differences encountered were probably due to the different kinds of nitrogen in the culture, as the effects of ammonium versus nitrate nitrogen were more pronounced on the chlorophyll content of the leaves than those of high versus low potassium.

2. Titrable acidity values reported as citric acid were greater, except in a few cases, in the high- than in the low-potassium cultures. Ascorbic acid values were greater in the low-potassium cultures of the nitrate-nitrogen series but smaller in the corresponding cultures of the ammonium-nitrogen series, indicating that ascorbic acid was affected more by the relations between nitrate versus ammonium nitrogen than by high versus low potassium.

3. Total sugar values were greater in the low- than in the high-potassium cultures, indicating a low rate of polymerization of sugars to starch or other complex carbohydrates in the former cultures. Sucrose as percentage of total sugars was greater in the high- than low-potassium cultures and was more abundant in the chlorophyllose than non-chlorophyllose tissues of the leaves.

4. Starch values were greater in the high- than in the low-potassium cultures, indicating a higher rate of synthesis in the former cultures, pre-

sumably from sugars. Starch depositions were greatest in the transitional and low chlorophyllose sections of the leaves and medial stem sections.

5. Hemicelluloses and celluloses plus lignin were slightly higher in the low- than in the high-potassium cultures.

6. These data emphasize that adequate amounts of potassium in the nutrient solution and consequently in plant tissues are essential for the condensation of reducing sugars to sucrose and starch.

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WATER RELATIONS OF POCOSIN OR BOG SHRUBS

MARY G. CAUGHEY

(WITH SIX FIGURES)

Introduction

The pocosins, or broad-leaved evergreen shrub bogs, constitute one of the most distinctive plant communities of the southeastern coastal plain. They occupy flat, poorly-drained areas where the soil is frequently water-logged for long periods of time, but where surface water seldom stands to a depth of more than a few inches, and then only for comparatively short periods. The water relations of the plants growing in pocosins present an interesting problem to the plant physiologist because structurally the leaves of many of the species resemble those of plants growing in a dry habitat rather than those of plants of wet places. The shrubs have simple, more or less elliptical leaves which are rather thick, leathery, and heavily cutinized. The leaf structure is of a type generally believed to have a low rate of transpiration, but there is never a lack of soil water except during severe summer droughts.

Though the problem of the water relations of bog plants has long been a subject for discussion, all of the earlier workers confined their investigations to northern bogs. SCHIMPER (**25**) was among the first to notice the discrepancy between leaf structure and habitat. He advanced the theory that bogs are physiologically dry, the absorption of water being hindered by the presence of certain humic acids and soluble salts released during decomposition of the organic matter in the soil. He believed that the structure of the bog sclerophylls materially aided in preventing excessive water loss from plants whose root systems were unable to absorb much water because of the physiological dryness of the habitat. This theory of physiological drought was more or less generally accepted. Most of the earlier workers merely speculated as to the cause of the alleged physiological drought, however, and failed to present much real experimental data on the actual water relations of the plants.

A number of causes of physiological drought have been suggested. Low soil temperature was regarded by SCHIMPER (**25**), WARMING (**30**), and TRANSEAU (**27**) as a factor in northern bogs. DAVIS (**13**) and BURNS (**3**) ascribed the "xerophytic nature" of bogs to the water-retaining power of the peat and to periodic droughts. TRANSEAU (**27**), CLEMENTS (**6**), and BERGMAN (**2**) regarded the lack of oxygen in bog soils as the controlling, or at least the primary, factor in physiological dryness. The importance of toxic compounds was first suggested by LIVINGSTON (**17, 18**) and has been advocated by DACHNOWSKI (**9, 10, 11**) and RIGG (**23**). These so-called toxic compounds are supposedly the result of incomplete decomposition of organic substances in a soil deficient in oxygen.

The hypothesis of physiological drought, originally developed in connection with bogs of cool climates, has been extended to explain the vegetative

type characteristic of southern bogs or pocosins. In the southeastern coastal plain, obviously, low soil temperature could not be a factor in producing physiological drought. WELLS (**31**) stated that the soil toxin theory furnishes the most probable explanation of the supposed physiological dryness of the southern pocosin. According to WELLS (**32**), the low soil oxygen in a bog habitat during periods of flooding interferes with root growth and absorption. He suggested that, although no transpiration data are available for bog shrubs, there could be little doubt that the coriaceous leaves of these plants protect them against excessive water loss, enabling the poorly developed root system, in its poorly-aerated environment, to maintain the proper water balance. WELLS and SHUNK (**33**) believe that this low oxygen content is responsible for a complex of soil factors in grass-sedge bogs which prevent other species from entering the area for several years after drainage. DACHNOWSKI-STOKES and WELLS (**12**) suggested that this "lag effect" in grass-sedge bogs and a similar effect which they observed in a pocosin may be attributed to high acidity or possibly to organic toxins.

A few investigators, among them MONTFORT (**21**) and STOCKER (**26**), have rejected the theory of physiological drought of the habitat and of low transpiration rates of typical bog plants. MONTFORT, in experiments on guttation and exudation from cut stems, found that bog water had little effect upon absorption of water by plants and therefore concluded that the bog habitat was not physiologically dry. STOCKER's investigations revealed that, per unit of leaf area, the transpiration rate of evergreen heath plants of northwestern Germany was between that of xerophytes and mesophytes, and the rate of deciduous species was equal to that of mesophytes or hydrophytes. He also calculated the ratio of daily transpiration to the fresh weight of the roots and concluded that bog plants absorbed more water per unit of root surface than did mesophytes.

The water relations of shrubs characteristic of bogs is, therefore, a controversial question, and no data are available for species of southern bogs. In order to clarify our knowledge of the water relations of these plants, a series of experiments was performed to compare the transpiration rates of pocosin species with those of certain mesophytes of the southeastern coastal plain. Since a knowledge of the environmental factors of a habitat is essential to a complete understanding of plant behavior and since no such data were available for a pocosin, a study of the habitat was carried out simultaneously with the transpiration experiments. It was hoped that such a study might furnish information which would aid in explaining the so-called xeromorphic leaf structures of these plants and lead to a better understanding of their water relations. The effect of certain of these environmental factors on the transpiration rates of these species was determined by experiments in the field and in the greenhouse.

Habitat study

A study of the environmental factors of both a pocosin and a nearby upland was made. First, because plants of both habitats were to be used

in the transpiration experiments; second, it was hoped that such a comparative study would lead to a better understanding of the factors in the bog habitat which are directly or indirectly involved in the water relations of these plants. A pocosin located in Beaufort County, North Carolina, about one-fourth mile north of the Craven County line, on U. S. highway no. 17, and an adjoining well-drained upland forest, representing a mesic habitat, were selected as sites for the field investigations. The most common shrubs in the pocosin were: *Clethra alnifolia* L., *Cyrilla racemiflora* L., *Gordonia lasianthus* L. (Ellis), *Ilex glabra* (L.) A. Gray, *Ilex lucida* (Ait.) T. and G., *Magnolia virginiana* L., *Myrica cerifera* L., *Persea pubescens* (Pursh) Sarg., and *Zenobia cassinifolia* (Vent.) Pollard. The dominant species in the upland were *Pinus palustris* Mill. and *Pinus taeda* L. Numerous hardwood species and several pocosin shrubs made up the understory.

Since the available soil water and the relative humidity of the atmosphere are probably the most important environmental factors affecting the transpiration of a given plant, the data reported in this paper bear on these factors only.

Continuous records of air and soil temperature, relative humidity, evaporation, and rainfall were obtained from March, 1940 to August, 1941. All the recording instruments were of the standard Friez type. Since the data obtained were too voluminous for complete tabulation, only summaries for weekly periods during the spring and summer of 1941 are reported in this paper. Compared with available records of the United States Weather Bureau for the nearby town of Washington, North Carolina, these data indicate that the period reported is typical of average conditions with respect to temperature and rainfall.

Minimum relative humidity values as recorded in the middle of the day and rainfall in inches are shown in figure 1. It is believed that the minimum relative humidity of the two sites is more important than the average or maximum humidity in respect to plant growth; therefore, only these values are reported. The curves show that minimum relative humidity in the pocosin was usually higher than in the upland, but the greatest variation between the two sites was only 10 per cent.

Average evaporation per week from four standard white bulb atmometers at each station is shown in figure 2. During the entire season evaporation rates were considerably lower in the pocosin than in the upland. A difference was to be expected since the relative humidity of the air in the pocosin was higher than in the upland. Some weeks there was approximately 40 per cent. more evaporation in the upland than in the pocosin. Although no records of wind velocity were obtained, repeated observations indicated that there was considerably more wind in the upland than in the pocosin. This difference in air movement may partially account for the greater variation in evaporation than in relative humidity in the two sites.

There was very little difference between air temperatures in the pocosin and at the upland station, and the same was true of soil temperatures. This

is not surprising since the two habitats were within a few hundred yards of each other. It seems probable that the small differences recorded were of little significance with respect to plant behavior, and they are, therefore, not included in this paper.

The pocosin soil consisted of a layer of organic matter approximately five to seven inches thick, overlying a sandy soil. This humus layer was composed of shrub leaves and sphagnum in various stages of decay interlaced with numerous fine roots and formed a fibrous mat over the underlying

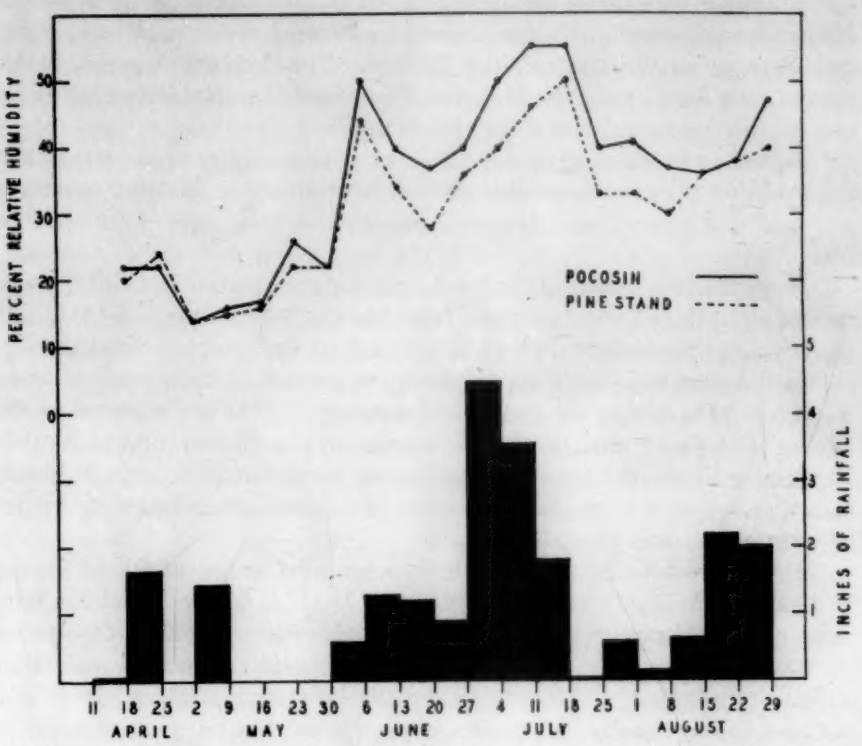

FIG. 1. Minimum relative humidity in the pocosin and pine stand and rainfall in inches (1941).

sand. The surface of the bog was made very irregular by numerous hummocks which varied from a few inches to 12 or 15 inches in height in the wettest parts. With the exception of sphagnum, the vegetation was largely confined to these hummocks. The floor of the upland was very different from the pocosin since it was practically bare of litter. This was the result of frequent fires and the rapid decomposition of organic matter in the hot humid climate of the region. The surface layer, six or eight inches in depth, was similar to the sandy layer beneath the peat in the pocosin.

The shallow root systems of the pocosin species were largely confined to the fibrous peat mats and, as far as could be ascertained, only a few roots

penetrated beneath the peat into the mineral soil. There was a deeper distribution of roots in the mineral soil of the upland, but even in this site, most of the roots were very close to the surface. The soil in both habitats was acid in reaction, but that of the peat was much more acid with a pH value of 3.5.

The level of the water in the pocosin soil varied only a few inches during the spring and summer of 1941, the variations being related to the amount of rainfall. From May 9 to June 6 there was only a half inch of rain, and the water level at the instrument shelter dropped from the surface of the peat in the depression to approximately four inches beneath the surface. Eight inches of rain fell between June 27 and July 11, temporarily raising the water level in all parts of the pocosin. In the wettest part of the pocosin,

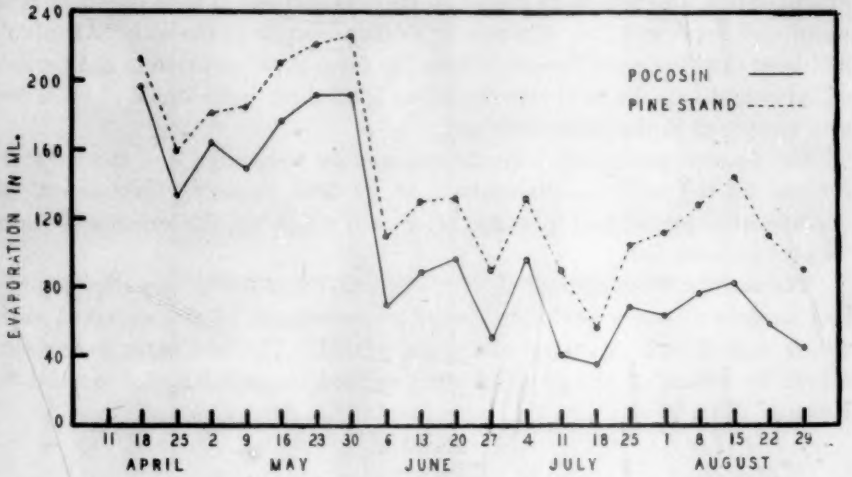

FIG. 2. Average evaporation per week from atmometers in upland and pocosin (1941).

water stood during the spring and summer several inches deep in the depressions between the hummocks. Nowhere in the pocosin was the water level ever more than 6 inches below the surface. Since water could always be squeezed out of it, the peat soil was considered always to contain readily available water for plant growth.

Although these data do not furnish a description of the habitat very different from that which might have been obtained by generalizations from careful observations, the writer believes that they, nevertheless, are of considerable value. They furnish the first exact measurements of some of the environmental factors in a pocosin, and with these specific data it is possible to interpret more accurately the relation between the environment and plant behavior.

Phytometer studies

These experiments are presented in four parts. The first concerns a comparison of transpiration rates in the two habitats. The second is a com-

parison of transpiration rates of pocosin species and mesophytic species. The third deals with the effects which flooding the soil has on transpiration rates. The fourth concerns the effects on transpiration which are produced by saturating the soil with carbon dioxide.

Materials and Methods

The plants studied were *Clethra alnifolia* L., *Gordonia lasianthus* L. (Ellis), *Ilex glabra* (L.) A. Gray, *Liriodendron tulipifera* L. (tulip poplar), *Myrica cerifera* L., *Pinus taeda* L. (loblolly pine), *Quercus alba* L. (white oak), and *Quercus borealis* var. *maxima* (Marsh) Ashe (eastern red oak). All of the pocosin species were potted in sandy soil obtained at the pocosin. The mesophytes were grown in the sandy loam used as potting soil in the greenhouse. The pots were placed in buckets and sealed to make them rainproof and to prevent loss of water by evaporation from the soil. All plants had been growing out-of-doors in pots for several weeks prior to the periods of investigation. In each experiment at least three individuals of each species were used in the determinations.

The transpiration rates were determined by weighings, and the moisture content of the soil was maintained at its field capacity throughout the experimental period by replacing, after each weighing, the amount of water which had been lost.

The results are expressed as grams of water lost per square decimeter of leaf surface or, on a per-plant basis, as percentage of the expected rates which were determined prior to the test period. The leaf areas were determined by means of the photo-electric method, modified and described by Kramer (**15**).

Results

Comparison of transpiration rates in the two habitats

If the pocosin is physiologically dry, there must either be some factor in the plant or in the environment which retards excess loss of water from the plant, and thus enables it to survive under such conditions. This experiment was devised to determine whether or not the atmospheric factors in a pocosin, as compared to those in the upland site, might limit transpiration sufficiently to be of any real protective value to the plant if its roots are in a physiologically dry habitat. The soil was maintained at or near field capacity, and, therefore, the important variable factors in the experiment were the atmospheric differences in the two sites.

Three typical pocosin species, Gordonia, Clethra, Myrica, and one mesophytic species (white oak) were used to compare transpiration rates at the two habitats. Four individuals of each species were arranged at random at each station. The containers were placed on the ground and covered with excelsior to prevent excessive heating of the soil by the sun. The transpiration rates for two-day periods were determined by weighings. The amount of water lost, expressed in grams per square decimeter of leaf surface, is shown graphically in figure 3.

A statistical analysis of the data showed that there was no significant difference between the transpiration rates of plants in the two habitats.

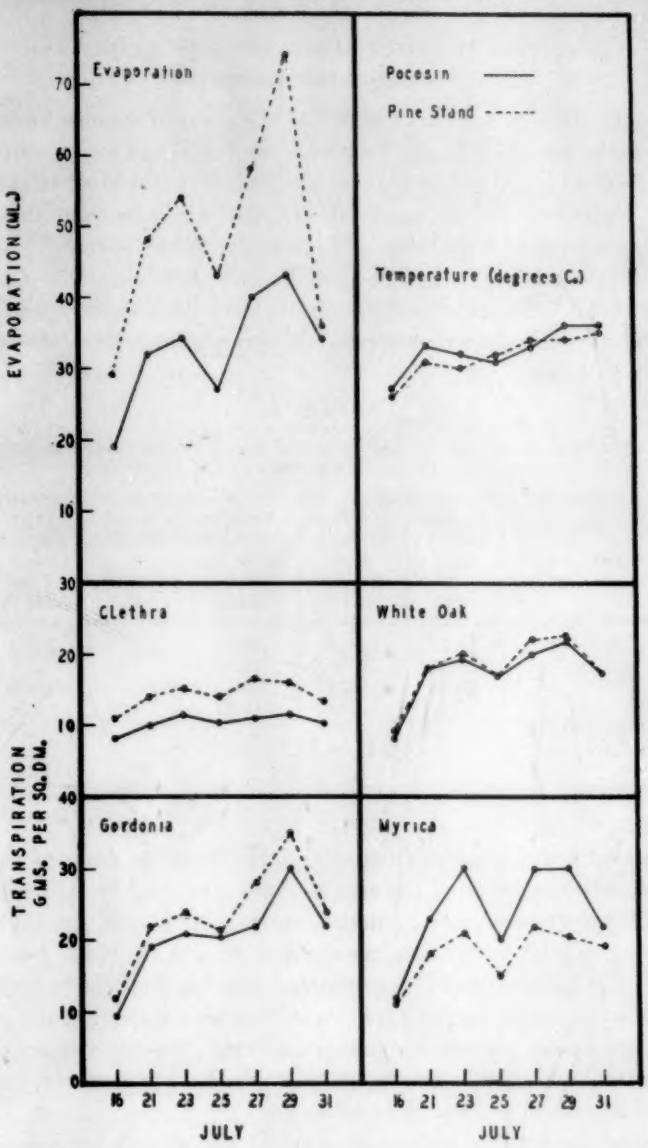

FIG. 3. Graphs showing transpiration of three pocosin shrubs and one mesophytic forest species in the pocosin and upland, also temperature and evaporation rates in the two habitats (1941).

About ten inches of rain fell during the three-week period prior to the beginning of the investigation, raising the water table in the pocosin to an unusually high level. High air temperature and high relative humidity

prevailed during the entire experimental period. If transpiration of pocosin plants is limited to any great extent by the atmospheric factors of the habitat, it certainly should have been evident during this hot, humid period.

Comparison of transpiration rates of pocosin and mesophytic species

The thick, leathery leaves of many of the pocosin species have led some investigators to assume that the leaves are well adapted to prevent excessive water loss, but no quantitative data on the transpiration rates of these shrubs have been reported. In this study the transpiration rates of three pocosin species were compared with those of four native forest species.

Three investigations were conducted out-of-doors during August and September, 1940. The experimental plants were the pocosin species Clethra, Ilex, and Myrica and the forest species tulip poplar, loblolly pine, white oak, and eastern red oak.

TABLE I

Comparison of transpiration rates of pocosin and forest species. Rates in gm./dm.^{2} day

Species	Experiment I; Aug. 1–10, 1940	Experiment II; Aug. 20–23, 1940	Experiment III; Aug. 24–Sept. 3, 1940	July 16–31, 1941 (Part I)	July 12–20, 1942 (Part III)	Average
Ilex glabra	19.22	16.10	16.81	.....	16.25	17.09
Myrica cerifera	12.31	10.80	10.36	9.17	.....	10.66
Clethra alnifolia	11.45	9.73	9.43	7.27	15.56	10.69
Quercus alba	11.34	14.21	13.21	9.03	14.58	12.27
Q. borealis var. *maxima*	.....	12.02	.....	.....	.....	12.02
Liriodendron tulipifera	.....	11.78	.....	.....	.....	11.78
Pinus taeda	.....	4.65	.....	.....	.....	4.65
Gordonia lasianthus	.....	.....	.....	11.96	23.58	17.77

Experiment I was a comparison of the transpiration rates of the pocosin species and white oak during the period from August 1 to August 10. Experiment II was a comparison of the transpiration rates of the three pocosin shrubs and the four forest species during the period from August 20 to August 23. In Experiment III, conducted from August 24 to September 3, different individuals of the four species included in Experiment I were used in the investigation. A summary of these three experiments together with some other transpiration data reported more in detail under Part I and Part III of this section is given in table I.

An inspection of table I shows that all of the pocosin species had transpiration rates comparable to those of the broad-leaved forest species. In fact, Ilex and Gordonia had a higher rate of transpiration than any of the forest species; and Myrica and Clethra showed rates very similar to those of white oak, eastern red oak, and tulip poplar. Clethra had the lowest rate of the pocosin species, and loblolly pine had the lowest rate of the forest species. However, the transpiration rate of Clethra was about 60 per cent.

higher than that of pine. These results clearly show that with similar conditions typical pocosin species transpire as readily per unit of leaf surface as the mesophytic species of the region.

Effects of flooding the soil on transpiration rates

It is well known that pocosin plants have root systems which are capable of surviving for comparatively long periods of time in poorly-aerated, waterlogged soil. The question naturally arises as to the effect of submergence on water absorption of these species. Two experiments were performed, one in the summer and the other in the fall of 1942, to determine the effects on transpiration of flooding the soil.

The first experiment, conducted out-of-doors in July, included Gordonia, Clethra, Ilex, and eastern red oak. The roots were constantly submerged

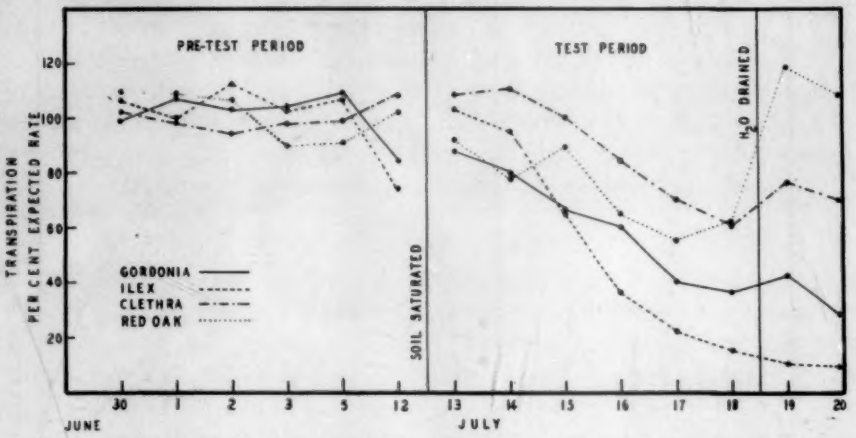

Fig. 4. Transpiration rates during the summer of 1942 as affected by water saturation of the soil. Soil was flooded on July 12 and drained on July 18.

from July 12 until July 18, at which time the excess water was drained off. The results are shown as percentages of the expected rate per square decimeter of leaf surface in figure 4. At the end of six days of submergence the rate of transpiration had decreased to less than 10 per cent. of the expected rate in Ilex, to 36 per cent. in Gordonia, 60 per cent. in Clethra, and 55 per cent. in eastern red oak. Foliage injury occurred in Ilex and Gordonia and it was necessary to drain off the free water on July 18. Clethra and red oak showed no visible signs of injury during this period.

A noticeable recurrence of growth was noted in both Ilex and Gordonia after the beginning of the experimental period. Since both the control and the test plants, as far as could be judged, showed approximately the same amount of growth, it was thought that no great error existed in the results obtained. The young growth at the tips of the branches of Ilex and Gordonia showed visible wilting on July 17 and 18. At the same time serious injury of numerous older leaves took place in both species. Because foliage

injury and low transpiration continued even after the excess water had been drained from the soil, it was necessary to terminate this experiment on July 20. It seemed probable that the serious injury occurring in Ilex and Gordonia might have been partially lessened had there not been a recurrence of growth at the time of complete submergence of the roots.

The submergence of the roots came simultaneously with a period when the air temperatures rose to nearly 38° C. and continued at that high level for approximately ten days. It was impossible to keep the temperature of

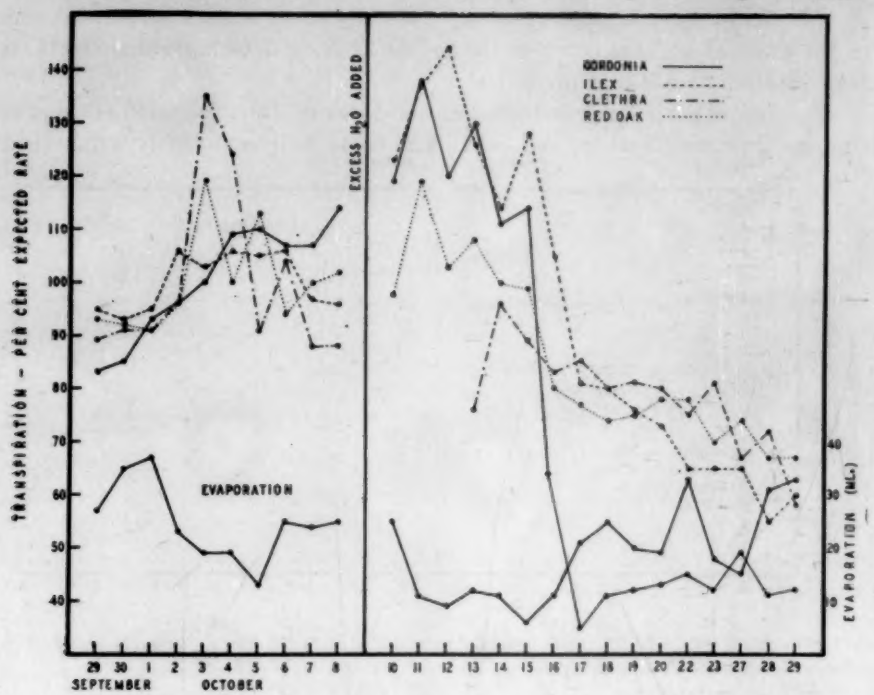

FIG. 5. Showing effects of water saturation of the soil on transpiration during the autumn of 1942. Soil was flooded on October 9.

the soil inside the containers below these high air temperatures. It seems unlikely that the temperature of bog water and soil is ever this high since the highest soil temperature recorded during the period of observation was 28° C. CANNON (4) says that roots require more oxygen for growth at high than at low temperatures. Probably poor aeration is much more injurious at high than at low soil temperatures. In this experiment it seems possible that a high oxygen requirement, at a time when the oxygen supply was greatly reduced, was partly responsible for the inability of the roots to supply the tops with sufficient water.

The second experiment was performed in the greenhouse during the fall of 1942. The soil of the test plants was flooded on October 9, and the roots were continually submerged until the termination of the experiment on

October 29. The results are given in figure 5 as percentages of the expected rates of transpiration on the basis of the average rate of four individuals of each species.

Although transpiration rates were reduced, there was no evidence of injury to any of the plants during the twenty-day period of submergence of the roots. The greatest reduction in transpiration occurred in Gordonia on October 17, with 35 per cent. of the expected rate; and Ilex came next with a reduction to 55 per cent. of the expected rate on October 28. The results of the two experiments indicate that those plants, which tend to have a relatively high transpiration rate under normal conditions, are more seriously injured and show a greater reduction in transpiration than those plants with a lower rate of transpiration. In table I it is shown that Ilex and Gordonia have relatively high transpiration rates, and in both of the experiments involving submergence of the roots they were more seriously affected than any of the other species.

These results indicate that transpiration of pocosin species is reduced by flooding the soil, even for relatively short periods, but that there is no serious injury except when flooding occurs at the same time as high air temperature and high soil temperature. During the milder autumn days, even after 20 days of submergence, there was no evidence of injury.

Effects of carbon dioxide in the soil on transpiration

Water absorption, by roots of pocosin plants growing in water-logged soils, is probably affected not only by lack of oxygen in the soil but also by a high concentration of carbon dioxide. Russell and Appleyard (**24**) have shown that the air dissolved in water-logged soils consists chiefly of carbon dioxide and nitrogen and contains practically no oxygen.

Kossowitch in 1892 was, according to Clements (**5**), the first to show that carbon dioxide exerted a specific effect on transpiration regardless of the presence of oxygen. More recently Kramer (**16**) stated that this rapid reduction in water intake by transpiring plants, caused by high concentrations of carbon dioxide, resulted largely from decreased passive absorption caused by physical changes in the protoplasm of the root cells. He said that high concentration of carbon dioxide is probably the most important factor in the reduction of water intake for the first few hours, but lack of oxygen is probably the most significant factor later. Whitney (**34**) believes that oxygen deficiency is more important than an excess of carbon dioxide in reducing water absorption. Chang and Loomis (**5**) reported that high carbon dioxide decreased absorption of water and minerals by wheat, maize, and rice.

An investigation to determine the extent to which transpiration of certain pocosin species may be reduced by high concentrations of carbon dioxide was conducted out-of-doors in October, 1941. The species studied were Ilex, Myrica, Clethra, and white oak; three individuals of each species were used as check plants and three individuals as test plants. Prior to the test period

transpiration rates of the six individuals of each species were determined in order to calculate the percentages of the expected rates. The soil was saturated with carbon dioxide from October 6 to October 11. Following the test period determinations were continued for five days in order to ascertain the rate of recovery. The average transpiration rates for the three individuals of both the test and the control plants were used to calculate the percentages of expected rate. The results are plotted in figure 6.

The curves show that transpiration rates were reduced in all species by the end of the first 24 hours of treatment. Ilex and Clethra were the least

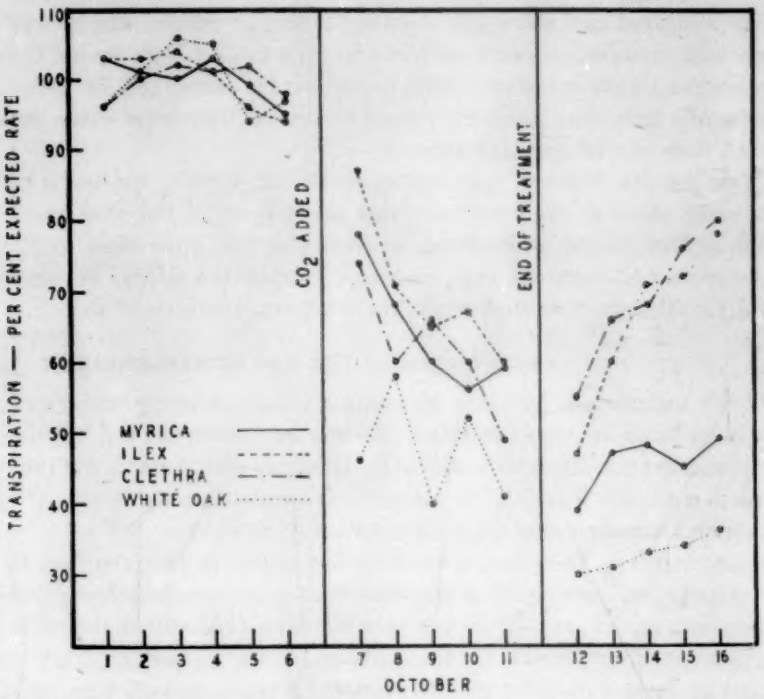

FIG. 6. Transpiration rates as affected by saturation of the soil with carbon dioxide (October, 1941).

affected during the five-day period. Transpiration was reduced about 50 per cent. of the expected rate in Clethra, 55 per cent. in Ilex, 60 per cent. in Myrica, and 70 per cent. in white oak. On the fifth day after the treatment was discontinued, Clethra and Ilex had recovered to approximately 80 per cent. of their normal rates, while Myrica had recovered to only about 50 and white oak to 35 per cent. of their normal rates. Two of the three individuals of the white oak receiving the treatment died within a week after the experiment was discontinued, but none of the pocosin species was visibly injured by the severe carbon dioxide treatment.

The period of investigation had clear skies, high temperatures, and high evaporation rates as measured by atmometers and was, therefore, quite

favorable to high transpiration rates. It is believed that if pocosin species are especially sensitive to the injurious effects of carbon dioxide, visible symptoms would have developed during a period so favorable to high transpiration. Therefore, although these pocosin species show reduction in transpiration when their roots are growing in soil containing appreciable amounts of carbon dioxide or in water-logged soil, they appear to be more tolerant of such conditions than species such as white oak and eastern red oak, which normally grow only in well-aerated soil.

Discussion

The data as shown by the curves in figure 3, reveal that transpiration in the pocosin is not significantly different from that in the upland forest. This might seem surprising since the relative humidity in the pocosin was found to be higher than in the upland. The phytometers in the pocosin probably received more direct sunlight than those in the upland although no direct measurements were made. In the pocosin there were only a few scattered pines, whereas, in the upland, the pines and the few hardwood understory trees furnished considerable shade. Although the shrub cover in the pocosin was dense, and an area had to be cleared to place the phytometers, the experimental plants were not shaded as much as were those in the upland. It appears, then, that the shrubs normally growing in the pocosin would receive more sunlight than similar shrubs growing in the upland. It was impossible to estimate to what extent, if any, this difference in amount of sunlight affected the transpiration rates at the two sites. Since the atmospheric factors are the only known variables in this experiment it seems logical to conclude that the atmospheric factors in the bog are not effective in limiting transpiration. This being true, if the plants are growing in a physiologically dry habitat the plant itself must limit transpiration sufficiently to balance the low water intake.

The data in table I furnish no evidence to support the view that the coriaceous leaf structure of these species is an adaptation enabling the plants to survive physiological or physical drought by virtue of low transpiration rates. The phytometer studies conclusively show that the pocosin species, Clethra, Gordonia, Ilex, and Myrica, growing in fairly well aerated soil with moisture content near the field capacity, have transpiration rates at least as high as those of such mesophytes as white oak, eastern red oak, and tulip poplar. In fact, Gordonia and Ilex consistently had higher rates than those of any of the forest species.

These data, of course, do not prove that the plants are not drought-resisting forms, but they do indicate that their leaf structure does not prevent water loss. MAXIMOV (19) has pointed out that not all drought-resisting plants have low transpiration rates but that actually many have comparatively high rates of transpiration when there is plenty of available soil water. It has been shown by TURRELL (28) that some plants having so-called xeromorphic leaves actually possess a high ratio of internal to

external surface that makes them capable of relatively high transpiration rates.

An anatomical study of the leaves of several pocosin species by McMenamin (**20**) has revealed the relatively large amount of surface exposed to internal air spaces. In *Gordonia lasianthus* the palisade is made up of two or three moderately compact layers and the spongy layer contains many air spaces. In *Ilex glabra*, in spite of the compact appearance of the palisade, there is a vast exposure of internal surface as revealed by sections cut parallel to the epidermis. Although the palisade layer is somewhat compact in *Clethra alnifolia* and *Myrica cerifera*, both possess comparatively open spongy tissue. Turrell (**28**) cited several experiments which showed sun leaves and leaves with much palisade to have higher transpiration rates than shade leaves. Turrell (**29**) also said that experimental plants, in his studies of the correlation between internal surface and transpiration rate, showed a high degree of correlation between the ratio of internal to external surface and the rate of transpiration. Since the amount of surface exposed to internal air spaces is an important plant factor in determining transpiration, it is not surprising to find that the pocosin shrubs used in this study had transpiration rates comparable to those of mesophytic species.

It is reasonable to suppose that transpiration rates obtained with phytometers are representative of the rates of shrubs growing in the pocosin under the same conditions. The root systems of the plants involved in these studies were certainly no more extensive, and probably much less extensive, in proportion to the tops, than similar systems in the peat layer of the pocosin. Under conditions of adequate moisture and in fairly well-aerated soil the roots of the experimental plants apparently were capable of supplying the aerial parts with relatively large amounts of water. The evidence indicates that the alleged xeromorphic leaf structures of the pocosin plants really do not retard transpiration.

Figures 4, 5, and 6 show that the absorption rates of pocosin species are reduced by excessively poor aeration. Injury to the plants is greatest when periods of poor aeration coincide with high air temperatures which favor rapid transpiration rates. However, there are several reasons which seem to indicate that situations comparable to the severe treatments to which the experimental plants were subjected are rarely, if ever, duplicated in the natural habitat. The water level, in the pocosin under investigation, fluctuated considerably during the period of observation. At certain seasons water stood to a depth of several inches in the depressions between the hummocks; but during June, July, and August, the water table was, with few exceptions, several inches below the surface of the peat in the depressions. The water table fell rapidly to a few inches below the surface during these months, even after having been raised considerably by heavy summer rains. Since the root systems of pocosin plants are mostly shallow and most of the shrubs grow on hummocks, the roots are in sphagnum and peat above the normal summer water table. Observations revealed that when extremely

wet periods occur during the growing season, many new roots develop at or near the surface of the peat above the high water level. Similar behavior was also observed in the potted plants, particularly Clethra and Myrica, after their roots had been submerged for several days.

It seems probable that the non-flooded peat layer is well aerated, although no determinations were made of the oxygen or carbon dioxide content of the soil-air. CLEMENTS (**7**) stated that, in VAGELER's (1907) studies of the soil-air of various moor communities, the greatest amount of carbon dioxide present was 2.68 per cent. and the least oxygen was 16.68 per cent.; no injurious effects from soil-air with a few per cent. of carbon dioxide was found anywhere in the moor. WELLS and SHUNK (**33**) reported that the soil-air in the non-flooded soil of a grass-sedge bog was similar to atmospheric air but that during a flooded period the oxygen content of mid-bog water was only about 0.3 p.p.m. EMERSON (**14**) stated that on account of the fibrous spongy structure of peat, aeration is possible down to the water surface, and he found that roots grow more normally in peat soil than in garden soil with the same water level. According to COVILLE (**8**) the necessity of well-aerated soil for the growth of blueberries accounts for their distribution either about the margin of bogs or on hummocks where, as he said, spaces in the sphagnum provide air passages even when it is saturated. Assuming, then, that the peat layer, above the water level, has an oxygen concentration at least comparable to that found in the soil of moor communities or in non-flooded, grass-sedge bogs, it may well be questioned whether inadequate absorption caused by poor aeration ever becomes a serious factor to plants in this pocosin. During weather favorable to high transpiration the water table is generally low enough to permit adequate aeration; and, when the water table is high, atmospheric factors are such as to limit transpiration. During the three summers' observation of this pocosin the water table never dropped more than six inches below the surface of the peat, and even then the peat was always so wet that water could be squeezed from it. One of these summers was a dry season, with a rainfall deficiency of five inches. It appears, therefore, that the shrubs in this pocosin would suffer from a deficiency of soil moisture only during the most severe droughts.

If we accept MONTFORT's (**21**) conclusion that absorption of water is not hindered by any toxic properties of the water or soil, and if aeration is not a problem during periods when water loss tends to be high, there seems to be no basis for considering the pocosin a physiologically dry habitat. STOCKER's (**26**) findings that the root systems of bog shrubs are more efficient in absorption per unit of surface than those of mesophytes, and our observation that some bog species have transpiration rates of the same magnitude as mesophytes do not support WELLS' (**32**) theory that the root systems of these plants are not capable of supplying any great amount of water to the tops. The water requirements of these plants apparently are high as indicated by the high transpiration rates and by their natural occurrence in bog habitats. That they have high oxygen requirements, at least during the

actively growing season, has also been demonstrated. The lack of aeration in bog soils apparently is compensated for largely by the shallow root systems and the growth of shrubs on hummocks in the wettest areas of the bog. The ability to survive in water-logged soil, when other plants cannot, may be due to their tolerance of high soil acidity. These shrubs probably are confined largely to the bog habitat more by their high water requirement than by any other factor.

The coriaceous leaf structure, characteristic of many pocosin species, cannot then be explained in terms of water relations. Some evidence points to the possibility that the characteristics of the vegetation of certain habitats are determined partly by mineral deficiencies. According to MOTHES (**22**), the coriaceous structures of bog plants may be attributed to a nitrogen deficiency and not to physiological dryness. ALBRECHT (**1**) suggested that lack of calcium in soils is correlated with a highly carbonaceous vegetation. The acid soil of the pocosin might be assumed, then, to favor the development of leaf structures with high carbon content which was described by MCMENAMIN (**20**) as characteristic of pocosin shrubs.

In conclusion, it seems that we probably need to revise our ideas about bogs and bog plants. Most discussions have been influenced by the somewhat archaic concepts of bogs as "physiologically dry" and of the plants growing in them as "bog xerophytes." It appears that these concepts have outlived their usefulness and could well be discarded. It will be possible to explain the physiology of the plants in our pocosins only after the accumulation of more experimental data, unhampered by traditional views.

Summary

A physiological and ecological study was made of a pocosin located in Beaufort County, North Carolina.

A comparison of the environmental factors of the pocosin and of an adjacent pine stand was made. Soil and air temperatures, as recorded by thermographs, are quite similar in the two habitats. Relative humidity is lower, and evaporation rates are considerably higher in the pine stand than in the pocosin. It is probable that the higher evaporation in the pine stand is caused partly by greater air movement in this site.

Phytometer studies indicate that transpiration rates at the two habitats are similar. The atmospheric factors do not appear to be sufficiently different in the two habitats to affect transpiration.

Soil factors of the pocosin and the pine stand differ more than do atmospheric factors. The accumulation of peat, the higher water table, and the greater acidity in the pocosin make its soil conditions quite different from those of the pine stand. The water level in the pocosin fluctuates considerably during the year. At certain times water stands to a depth of several inches in the depressions between the hummocks, and at other times it falls several inches below the surface of the peat. When the water level is high the roots of the shrubs may be submerged and thus subjected to low soil oxy-

gen, but when the water level falls the roots are in relatively well-drained, and probably well-aerated peat. Since most of the shrubs grow on hummocks it appears unlikely that the root systems suffer from inadequate aeration for any great length of time.

Phytometer experiments in which the soil was saturated with water or carbon dioxide indicate that transpiration of pocosin species is reduced as much by poor aeration as is transpiration of mesophytic species. The pocosin species, however, seem to be injured less by poor aeration of the soil than do mesophytes.

Observations extending over three years indicate that generally during periods when atmospheric factors favor high transpiration rates the shallow root systems are seldom submerged for more than a few days at a time. The problem of aeration, therefore, seems to be of less importance than has commonly been supposed.

Experiments show that the pocosin species *Clethra alnifolia, Gordonia lasianthus, Ilex glabra,* and *Myrica cerifera* have transpiration rates at least comparable to those of mesophytic forest species. The xeromorphic structures of the leaves of the pocosin shrubs apparently are not effective in reducing transpiration.

The pocosin habitat is probably not physiologically dry so far as the pocosin plants are concerned, and these plants are not xerophytes in the sense of having low transpiration rates. No clear explanation for their xeromorphic appearance can be offered.

The writer gratefully acknowledges her indebtedness to Dr. Paul J. Kramer for suggesting the problem and for aid and guidance throughout the investigation and to Dr. Kramer and Dr. H. J. Oosting for their critical review of the manuscript. The assistance of Mr. T. H. Wetmore in the care of the seedlings is greatly appreciated. The work was done at the Department of Botany, Duke University.

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BRIEF PAPERS

A NOTE ON THE EFFECTS OF COPPER IMPURITIES IN DISTILLED WATER ON GROWTH OF PLANTS

T. C. Broyer and A. H. Furnstal

The observation was made in certain controlled nutrient solution studies with sugar beet and barley, that toxicity was associated with the supply of distilled water. The toxic element was referred to copper. The condition arose from storage of the distilled water in tin-lined copper tanks in which areas of exposed copper were evident. Direct analysis, by the colorimetric sodium diethyldithiocarbamate method (**2**),[1] showed that the water issuing from the tin-lined copper still was relatively low in copper content, but increased in concentration the longer the water was retained in the defective reservoir. The approximate concentrations of copper which may be found in distilled water and artificial nutrient solutions are presented in table I.

TABLE I

COPPER CONCENTRATIONS COMMON TO DISTILLED WATER AND NUTRIENT MEDIA FOR PLANT GROWTH; GIVEN IN MICROGRAMS PER LITER

	γ
Distilled water (from glass, A)	Nil
Distilled water (A) plus repurified major nutrient salts	20
Distilled water (tin-lined copper still and reservoir, and tin pipe; B)	10–50
Distilled water (B) plus unpuried, major nutrient salts	50–80
Distilled water (the same as B except that areas of exposed copper were evident in the reservoir), *toxic*	170–200
Theoretical Cu in micro-nutrient supply (1)	20

A special study was instituted to determine the relation of the injury to the excessive copper concentrations in the nutrient media, using barley as a test species. This proved positive. The roots were particularly affected, characterized by a dark color and stunted appearance, early in the growth cycle. Prolonged injury affected shoot growth as well, evidenced by reduced growth. The older leaves died progressively, back from the tips. The toxicity was accentuated when the osmotic concentration of the nutrient medium was proportionally low (Cu proportionally high). The injury was relatively reduced in the presence of a balanced micro-nutrient supply; in other words, the toxicity was greater when other micro-nutrients (B, Mn, Zn, Mo) were restricted in their supply to the plants. Any condition leading to an excessive rate of absorption of copper may lead to injury; con-

[1] The copper concentrations normally dealt with in nutrient solutions approach the minimum for sensitivity with the reagent and technique employed (sensitivity about ± 0.01 mg. per liter). Other procedures, including the polarograph, are sensitive to about the same order of magnitude or greater. The mass spectrograph is more sensitive but impractical under some circumstances.

versely, any modification of conditions tending to reduce relatively the absorption of copper [which, within small concentrations is essential to vegetation (**3**)] per unit growth, will suppress or counteract the deleterious effects.

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NOTES

Report of the Executive Secretary-Treasurer.—The general summary presented herewith will serve to acquaint members with the state of the Society for the year ending July 1, 1945. It is gratifying to note the increase in memberships, subscriptions, and the total assets of the group; there is every indication of continued improvement during the current fiscal year.

The assets of the Society were increased by $906.05 during the past year. This accumulated in spite of the fact that five issues of PLANT PHYSIOLOGY were paid for during the fiscal year as well as Bulletin 15; increases in printing costs are also included in these considerations. In addition some expense has been incurred in establishing new Editorial and Executive offices and procuring supplies for the coming year. The general endowment cash account has been increased by $775.37 by virtue of income from securities and the sale of back numbers of the journal. There will probably be additional permanent investments made from the cash endowment fund during the current year.

The Society has enjoyed a healthy growth in membership with an increase of 40 for a total of 632 at this writing. Subscriptions have also increased to a total of 502; a gain of 74.

The total net worth of the Society as of July 1, 1945, was $18,031.84; of this amount $3000 is in savings and $10,000 represents permanent, endowment investments. Total receipts for the year 1944–45 (including the sale of two bonds) were $10,710.35; total expenditures (including the purchase of two bonds) were $9,804.30.

Dr. Edwin C. Miller.—After 35 years of service, DR. MILLER, Professor of Plant Physiology at Kansas State College and Plant Physiologist of the Kansas Agricultural Experiment Station, retired on August 31, 1945. On the evening of May 28 a Department dinner was held honoring Dr. and Mrs. MILLER, at which time administrative officers of the College and Experiment Station reviewed Dr. MILLER'S work. President M. S. EISENHOWER, of the College, announced that Dr. MILLER had been made Professor Emeritus of Plant Physiology. A book of more than 200 letters from former students, colleagues, plant physiologists, and other friends was presented to Dr. MILLER and a Department gift was presented to Dr. and Mrs. MILLER. The EDWIN C. MILLER FUND in Plant Physiology was announced. This loan fund of $200 was provided by his son, JORDAN Y. MILLER, and daughter, Mrs. JOSEPHINE MILLER HENDERSON, and her husband. Dr. MILLER'S address is R. F. D. #1, Baltimore, Ohio, where he retired to the farm on which he was born. Dr. JOHN C. FRAZIER, who has been associated with Dr. MILLER since 1936, assumed the teaching and research in Plant Physiology at Kansas State College and the Kansas Agricultural Experiment Station.

Leon Hatchig Leonian.—The American Society of Plant Physiologists mourns the passing of a distinguished member. Dr. LEONIAN was born in Van, Armenia, February 27, 1888; died in Morgantown, West Virgnia, June 7, 1945. His death followed an illness of over a year, but he remained active in his work until a few weeks before his death. Dr. LEONIAN was the son of HATCHIG and ANNA LEONIAN. He is survived by his widow, NELL LANHAM LEONIAN, three sons, PHILLIP, ARMEN, and JOHN.

He came to the United States at the age of twenty. He received the B.S. degree from the University of Kentucky in 1916; the M.S. degree from the University of Michigan in 1917; the Ph.D. from the latter institution in 1922. In that year he joined the staff of the College of Agriculture and Experiment Station, West Virginia University, as Assistant Plant Pathologist. At the time of his death he was Professor of Mycology.

His early scientific work was on plant diseases, but he soon became interested in the physiology of growth and reproduction of microorganisms, and for more than twenty years he was active in this field. His avocation was the breeding of choice delphiniums, day lilies, and oriental poppies. For many years he was editor of Delphinium.

This kindly man will long be remembered by his associates and his students. He was a good fighter for those things he considered right and just. A Student Loan Fund has been established in his memory by his colleagues and friends.

Photosynthesis and Related Processes.—Volume I, 1945, by EUGENE I. RABINOWITCH, published by Interscience Publishers, Inc., 215 Fourth Avenue, New York City, 599 pages. Price $8.50 per copy.

This publication fills the need for a comprehensive and critical compilation of recent researches of photosynthesis. The author has endeavored to correlate the more orthodox physico-chemical investigations of the process with the in vivo researches of plant physiologists. Critical examination is given data on the mechanisms which permit utilization of the large amounts of energy involved in photosynthesis and those processes which retard or prevent back reactions capable of destroying the unstable intermediates in sugar formation. The new experimental approaches to the study of carbohydrate synthesis developed during the past two decades are exhaustively presented, including oxygen liberation by isolated chloroplasts, the study of bacterial and algal forms, the use of flashing light, the rôle of isotopes, and study of accessory pigments. Special emphasis is given to researches published since 1925, thus obviating recapitulation of studies reviewed in the earlier monographs by STILES (1925) and SPOEHR (1926).

The work is divided into four parts, the first two of which are contained in volume one: 1. The Chemistry of Photosynthesis and Related Processes; 2. The Structure and Chemistry of the Photosynthetic Apparatus; 3. The Spectroscopy and Fluorescence of the Pigments; and 4. The Kinetics of

Photosynthesis. Volume II containing parts three and four is expected to appear within the coming year.

The wealth of data which the author has carefully assembled, ingeniously correlated, and critically evaluated makes this publication an outstanding contribution in its field. Though frequent reference to theoretical and speculative aspects of the problems of photosynthesis occasionally makes perusal difficult, they have obviously been included for purposes of comprehensive presentation and guidance of original investigators. The format of the book is excellent and the printing of the intricate formulae and equations is extremely accurate. Each chapter is supplemented with a bibliography arranged in chronological order. In addition to the usual subject index to the volume, there is an author index which cites the subject matter and page of the text on which the references occur.

Plants and Plant Science in Latin America.—1945, edited by FRANS VERDOORN, The Chronica Botanica Co., Waltham 54, Massachusetts, U. S. A. 384 double column pages. Price $6.00.

The aim of this collection of nearly a hundred descriptions of the vegetation and plant resources of the countries of Central and South America is to give the agronomist, botanist, forester, and phytopathologist information on the wild and cultivated plants of Latin America. The book will also be useful to those who plan to visit Latin America to collect or to conduct research. The publication endeavors to give information concerning the present status and needs for research in the chief branches of the pure and applied plant sciences in Latin America. In addition to data in his own field, the specialist will find much useful and stimulating information on vegetational and agronomic problems in general, on the organization of research, lists of books for consultation, addresses of institutions and societies in the territory in which interested.

The book also contains various chapters on the present agricultural problems of each of the Central and South American countries, including phytogeography, agricultural economics, soils, forestry, pathology and conservation. The special supplement listing various plant science institutions will be of particular value to scientific readers. This supplement contains a list of experiment stations, gardens, museums, agricultural societies, and commissions in Central and South America. Fairly comprehensive descriptions of the agricultural agencies are given among the various chapters of the book. This publication constitutes a very comprehensive compilation of the contemporary status of plant science and agriculture in Central and South America. The book has an excellent format for easy reading and rapid reference use. The table of contents is very complete and adds immensely to the general usefulness of the publication. An index of personal names further enhances the reference value of the book.

Injury and Death of Bacteria by Chemical Agents.—1945, by OTTO RAHN and edited by B. J. LUYET. Number Three of Biodynamica Monographs published by Biodynamica of Normandy 21, Missouri, U. S. A. 183 pages. Price $3.60.

This text by an eminent investigator of forty years' research experience in his specialty will be read with interest by those interested in the action of antiseptics and disinfectants. Arguments are advanced that survival curves of bacteria and other unicells are exponential and that injury and death result from the impairment or destruction of individual physiological functions by disinfectants. The author also attempts to systematize the currently confused study of substances which arrest or delay the activity of bacteria without killing them. The author's presentation of a new direct method for the evaluation of disinfectants rather by comparison with phenol is of particular significance.

Five chapters are devoted to problems of the Logarithmic Order of Death of Bacteria, and fifteen chapters to the Mode of Action of Disinfectants and Antiseptics. The discussion concludes with an excellent summary of the conclusions. A comprehensive bibliography without titles of original papers cited, as well as separate subject and author indices, are included.

Actinomycetes in Various Parts of the Potato and Other Plants.—1945, by B. F. LUTMAN. It may be obtained as Bulletin 522, Vermont Agricultural Experiment Station, Burlington, Vermont, U. S. A. 72 pages.

The presentation of a new and unorthodox interpretation of the stimulus to tuber formation will interest physiologists and agronomists. The author's data on the presence of actinomyces in cell walls is of general significance to phytocytology and the physiology of development, especially in relation to flower abscission and protein synthesis. If the author's viewpoint as to the widespread presence and rôle of actinomycetes in cell walls is confirmed, it will result in an entirely new concept of wall composition and function in plants.

Annual Review of Biochemistry.—Volume XIV, 1945, edited by JAMES MURRAY LUCK. Published by Annual Reviews, Inc., Stanford University, California, U. S. A. 856 pages. Price $5.00.

This volume is the largest of this well-known series to date. Each subject has been reviewed by research experts in the particular field. The more important recent research data have been well correlated and evaluated by the reviewers. Each topic is well documented with a comprehensive bibliography giving the names of authors, journal pages, and year but not the title of the original paper cited. The author index giving pages on which the original work is cited and the very complete cross-referenced subject index of the volume permit rapid reference. Though only the last five chapters deal especially with plant materials, many of the others also contain numerous data of botanical importance.

Trees and Toadstools.—1945, by M. C. RAYNER. Published by Faber and Faber, 24 Russell Square, London, England. 71 pages. Price 6s. The author has succeeded in presenting in an accurate, non-technical, and interesting way the regularly occurring phenomena of woods and pastures. The author, a recognized specialist in this field, has told comprehensively the story of the association of trees and fungi and the general occurrence of certain species of fungi with certain genera of trees. A discussion of the historical interpretations of the term "symbiosis" has added interest. Fine explanatory photographic plates are dispersed throughout the book.

Plant Growth.—1945, by L. EDWIN YOCUM. Published by Jaques Cattell Press, Lancaster, Pennsylvania, U. S. A. 203 pages. Price $3.00. This book is written for the layman who wishes a more scientific concept of the plant in relation to its environment. The book discusses the morphology of the plant and its development physiologically. Chapters are devoted to the germination of seeds, absorption of water and mineral salts, the soil, soil improvement, propagation, pruning and training of plants, and plant hormones. Heredity and variation are presented with an historical account of Mendel's work and the work done later with hybridization. Besides the index, references are included at the end of each chapter, and a glossary of terms is presented for easy reference.

Meeting of AAAS and ASPP.—There will be a spring meeting of the American Society of Plant Physiologists in connection with the American Association for the Advancement of Science at St. Louis, Missouri, March 27 to 30 inclusive. Announcements of program and call for titles will soon be mailed to members of the ASPP.

Errata.—Attention of members and subscribers is called to the list of errata at the close of the table of contents of volume 20. We regret the errors and suggest that corrections be entered at once in order to avoid future misinterpretations.

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